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Beta blocker specificity: a building block toward personalized medicine.

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Drugs known as beta blockers, which antagonize the β-adrenergic receptor (β-AR), are an important component of the treatment regimen for chronic heart failure (HF). However, a significant body of evidence indicates that genetic heterogeneity at the level of the β1-AR may be a factor in explaining the variable responses of HF patients to beta blockade. In this issue of the JCI, Rochais et al. describe how a single amino acid change in β1-AR alters its structural conformation and improves its functional response to carvedilol, a beta blocker currently used in the treatment of HF (see the related article beginning on page 229). This may explain why some HF patients have better responses not only to carvedilol but to certain other beta blockers as well. The data greatly enhance our mechanistic understanding of myocardial adrenergic signaling and support the development of “tailored” or “personalized” medicine, in which specific therapies could be prescribed based on a patient’s genotype.

Beta blocker specificity: a building block toward personalized medicine

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Beta-AR polymorphisms and cardiac phenotype

The β-ARs are members of the G protein-coupled receptor (GPCR) superfamily, which consists of over 700 genes that are the targets of more than 50% of the drugs in clinical practice (2). There are 3 known types of β-ARs, β1, β2, and β3. The β-ARs are located mainly in the heart, kidney, and adipose tissue. The β2-ARs are located mainly in the heart, lung, gastrointestinal tract, liver, pancreas, and skeletal muscle. The role and location of β-ARs are less well defined. When stimulated by agonists, β-ARs primarily activate heterotrimeric guanine nucleotide–binding (G) proteins of the Gi family, causing dissociation of GαGTP and Gβγ subunits. The G proteins transduce intracellular signaling pathways via adenyl cyclase (AC) activation, and this results in increased intracellular cAMP levels (3). This signaling cascade ultimately leads to positive regulatory input to the myocardial contractile apparatus. Importantly, β-ARs present on cardiomyocytes represent the most powerful means of enhancing the contractile performance of the heart.

The predominant β-AR subtypes of functional consequence to cardiac physiology are β1-AR and β2-AR, both of which have...
well-documented polymorphisms that exist in human populations. The coding region of the gene encoding the β$_{1}$-AR, located on chromosome 10q24-26, contains 2 known SNPs resulting in amino acid substitutions. At position 49 in the extracellular amino terminus of the receptor, a serine is substituted by a glycine (Ser49Gly) with an allele frequency of 0.87 and 0.13, respectively (4). At position 389 in the intracellular carboxy terminus in the proximity of the seventh transmembrane spanning segment, a glycine or arginine can be found at an allele frequency of 0.25 and 0.75, respectively (4). These polymorphic variants of the β$_{1}$-AR appear to have significant cardiovascular phenotypic consequences. For example, Ranade et al. found a significant functional association between the Ser49Gly polymorphism and resting heart rate, in which Gly49 homozgygotes had the lowest heart rate, and each Ser49 allele increased the basal heart rate in an additive model (5). The Arg389 variant appears to be linked with HF in both clinical outcomes and response to therapy (6).

More specifically, the Arg389Gly polymorphism lies within the putative Gs-binding domain of the β$_{1}$-AR (Figure 1), and consistent with this localization, previous in vitro studies in fibroblast cell lines transiently transfected with the Arg389 β$_{1}$-AR have demonstrated enhanced receptor-Gs coupling as

Figure 1
The effect of the β$_{1}$-AR Gly389Arg polymorphism on signal transduction and beta blockade by carvedilol. The Gly389Arg polymorphism of the β$_{1}$-AR occurs in the region between the seventh transmembrane domain and the intracellular tail of the receptor (R and G correspond to arginine and glycine, respectively, at position 389 of the receptor). This highly conserved region is putatively associated with coupling to the Gs protein. The change of the amino acid residue at position 389 of the β$_{1}$-AR from the polar, basic arginine (upper 3 panels) to the small, nonpolar glycine (lower 3 panels) may result in a modified structure that could alter receptor-Gs interaction. This region of the cytoplasmic tail of the receptor may participate in regulating the affinity of the receptor-Gs interaction. This could potentially explain the differences in receptor-Gs coupling and cAMP production associated with the Arg389 β$_{1}$-AR variant as compared with the Gly389 β$_{1}$-AR variant reported previously (7, 8). Under basal conditions, the Arg389 variant has been associated with increased cAMP production, which is markedly augmented as compared with the Gly389 variant following agonist stimulation (7). This effect could also be attributed to a more favorable structural environment for interaction with Gs. Carvedilol treatment induces a conformational change resulting in the intracellular tail of the receptor being positioned more closely to the third intracellular loop; this inverse agonism of the receptor in theory would lead to decreased Gs coupling and reduced cAMP accumulation. Both variants demonstrated inverse agonist properties in response to carvedilol; however, the effect was increased 2.5-fold in the Arg389 variant, apparently due to conformation of the Arg389 structure in the presence of the ligand as observed by Rochais et al. in this issue of the JCI (14).
β₁-AR signaling in real time

Recent advances in the fields of biochemistry and applied science have generated new classes of fluorescent probes to permit the direct assessment of the dynamic behavior of biological molecules. Specifically, fluorescence resonance energy transfer–based (FRET–based) techniques have allowed investigators to evaluate dynamic protein–protein interactions and protein conformational changes in real time (for reviews see refs. 12, 13). FRET fundamentally characterizes the spatiotemporal relationship of fluorescently labeled molecules within a system by way of recording the energy transfer from a donor fluorophore in an excited electronic state to an acceptor fluorophore of adequate proximity. In this issue of the JCI, Rochais and colleagues report on their exploitation of FRET technology to generate a β₁-AR FRET sensor to directly assess the effects of the Arg389Gly polymorphism in the β₁-AR on receptor activation, downstream signal transduction, and response to beta blockade in cultured human cells in real time (14). The β₁-AR FRET sensor used in this study was generated based on evidence that the receptor undergoes conformational changes upon agonist binding, specifically in the region of the carboxy terminus and third intracellular loop, which move apart upon receptor activation. The authors then tagged these regions with cerulean and yellow fluorescent proteins, respectively, allowing them to detect the movement of these regions in real time using FRET, thereby tracking the dynamic changes in the receptor in response to agonist and antagonist binding. The authors demonstrated that this β₁-AR FRET sensor shares pharmacological and signaling characteristics consistent with that of the WT β₁-AR, including similar activation kinetics and downstream signaling via cAMP formation.

Utilizing β₁-AR FRET sensors for both Gly389 and Arg389 variants of the β₁-AR, the authors were able to directly assess the functional consequences of β₁-AR polymorphisms with respect to receptor conformation in response to pharmacologic agonists and antagonists. In contrast with results in previous in vitro studies (7, 8), the authors identified no significant differences between Gly389 and Arg389 variants in β₁-AR–mediated Gs coupling or cAMP accumulation in response to agonist (14). Perhaps the most exciting clinically relevant data from this study is the comparison of the FRET responses of the 2 β₁-AR variants generated by the β₁-AR antagonists bisoprolol, metoprolol, and carvedilol. Each β-AR antagonist induced an increase in FRET ratio, signifying the occurrence of an active change in receptor conformation upon antagonist binding, suggesting an inverse agonist mechanism of action on the β₁-AR. Increases in FRET ratio were minor with respect to decreases in receptor activation for metoprolol and bisoprolol, and the effects of these 2 beta blockers did not differ between β₁-AR variants (14). In contrast, carvedilol induced strong inverse agonism with regard to both β₁-AR variants; furthermore, the Arg389 β₁-AR variant exhibited a 2.5-fold increase in FRET ratio as compared with the Gly389 β₁-AR variant. The unique properties of this beta blocker were confirmed downstream of receptor inactivation at the level of decreased cAMP accumulation: both β₁-AR variants demonstrated marked reduction in basal cAMP levels (14). Carvedilol led to a much stronger reduction in basal cAMP content in the Arg389 β₁-AR variant as compared with the Gly389 β₁-AR variant (see Figure 1). The authors further established the phenotypic relevance of the molecular alterations induced by the Arg389Gly polymorphisms using primary neonatal rat cardiac myocytes infected with adenoviruses containing the β₁-AR variants as a model system to study cardiac rate control. Under basal conditions, both β₁-AR variants induced an increase in contractile activity as compared with WT cardiac myocytes; however, the contractile activity of the Arg389 variant was increased nearly 1.5-fold as compared with the Gly389 variant, and carvedilol significantly decreased this activity preferentially in the Arg389 β₁-AR.

Overall, the study by Rochais et al. (14) represents the first direct assessment of GPCR function using the well-characterized β₁-AR system that also happens to be critical for normal and pathological cardiac physiology. Of importance, the authors were able to assess the conformational changes that occurred during agonism and antagonism of the receptor. Moreover, the data reveal that different β₁-AR antagonists, particularly carvedilol, are capable of inducing varying degrees of inverse agonism on the receptor and that this effect of carvedilol is dependent on the amino acid residue present at position 389 of the β₁-AR. Carvedilol, unlike metoprolol or bisoprolol, is a nonselective beta blocker and can also act on α₁-ARs (15). Therefore, it would be interesting to address the potential effects of β₂-AR and α₁-AR signaling on cardiac rate control to ascertain whether any receptor crosstalk or dimerization is responsible for the decreased basal...
Taking it personally

The implications of these findings are of potentially profound clinical importance when considering the interindividual and ethnic variation that occurs in response to β-AR blocker therapy in the treatment of HF. It has been reported that the allele frequency of the Arg389 variant is 20% less common in black patients compared with non-black patients, and this may partially explain the poorer response to β1-AR antagonists seen in blacks compared with that of the rest of the population (17). Future efforts to characterize the effect of genetic heterogeneity in cellular response to pharmacological agents, such as the β1-AR antagonists examined in this study, lend credence to the viability of personalized medicine for the Arg389 variant (Figure 1), and studies such as this could lead to research broadening our knowledge of this pharmacological property of GPCR ligands and therefore potentially increase their clinical utility (16).

Macrophage heterogeneity and tissue lipids

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Macrophages are present as resident cells in adipose tissue, and blood monocytes are recruited in increased numbers to sites of lipid accumulation in atherosclerosis, a modified form of inflammation in the arterial wall. Recent findings reported by 3 separate groups in this issue of the JCI provide evidence for distinct monocyte subsets, differential chemokine receptor usage, and phenotypic modulation of macrophages in murine models of genetic and high-fat diet–induced disease (see the related articles beginning on pages 175, 185, and 195). These studies raise prospects for selective therapeutic targets to ameliorate macrophage hyperinflammatory responses, while sparing host defense and repair mechanisms.

Monocytes and mature macrophages are prominent in the host response to lipid accumulation in major arteries, the development of atherosclerotic plaques, and their complications (1). Less established are the details of their colocalization and possible metabolic interactions with adipocytes in body fat stores (2). New studies reported in this issue of the JCI by Swirski et al. (3) demonstrate that a monocyte subset that expresses high levels of a marker antigen, Ly-6C, dominates hypercholesterolemia-associated monocytes and gives rise to macrophages in atheroma. Also in this issue, Tacke et al. (4) report that monocyte subsets differentially employ the chemokine receptors C-C motif chemokine receptor 2 (CCR2), CCR5, and C-X-C motif chemokine receptor 1 (CX3CR1, also known as the fractalkine receptor) to enter atherosclerotic plaques. They also exploited an mAb, Gr-1, directed against Ly-6 family antigens to distinguish monocyte subsets and used CD11c, a β2 integrin expressed by myeloid DCs and selected tis-

Nonstandard abbreviations used: CCR2, C-C motif chemokine receptor 2; CX3CR1, C-X-C motif chemokine receptor 1.

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