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Interaction Between Cardiac Sympathetic Drive and Heart Rate in Heart Failure

Modulation by Adrenergic Receptor Genotype

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OBJECTIVES In the present study, we aimed to evaluate the effect of adrenergic receptor polymorphisms on the response of myocardium to measured levels of cardiac adrenergic drive, and to evaluate whether polymorphisms of presynaptic adrenoceptors modified the rate of cardiac and systemic release of norepinephrine.

BACKGROUND Heightened sympathetic activity plays an important pathophysiologic role in congestive heart failure (CHF). Recently several functionally relevant polymorphisms of the α_2-, β_1-, and β_2-adrenoceptors have been identified, and specific genotypes have been associated with the incidence or clinical severity of CHF. These adrenoceptors are known to be located both pre-synaptically (α_2 and β_2) and post-synaptically (β_1 and β_2), raising the possibility that their association with clinical measures in CHF could be mediated either by modulation of the cardiac response to a given level of adrenergic drive or by altering norepinephrine release from sympathetic nerve terminals.

METHODS We determined the β_1-, β_2-, and α_{2C}-adrenoceptor genotype in 60 patients with severe CHF in conjunction with measurement of cardiac and systemic sympathetic activity using the radiotracer norepinephrine spillover method.

RESULTS We showed a strong relationship ($r = 0.67$, $p < 0.001$) between heart rate and the level of cardiac adrenergic drive, and heart rate for a given level of cardiac adrenergic drive was substantially greater in patients with the Arg/Arg16 β_2-adrenoceptor polymorphism ($p = 0.02$), whereas no such relationship existed for polymorphisms of the β_1-adrenoceptor. The genotype of the α_{2C}- and β_2-adrenoceptors showed no relationship to the rate of norepinephrine release from cardiac sympathetic nerves.

CONCLUSIONS For the first time, we show that β_2-adrenoceptor polymorphisms significantly influence the relationship between heart rate and cardiac adrenergic drive in CHF, but do not affect the rate of norepinephrine release from sympathetic nerve terminals. (J Am Coll Cardiol 2004;44: 2008–15) © 2004 by the American College of Cardiology Foundation

An extensive body of evidence based on clinical trial data and human and animal neurochemical studies supports the concept that congestive heart failure (CHF) is characterized by heightened sympathetic tone and that this exerts an adverse influence on the progression and outcome of CHF (1–3). Despite the relative uniformity of these observations,

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many issues with regard to the biology of the sympathetic nervous system and the pharmacologic approaches that are available to manipulate its activity (directly or indirectly) remain unresolved. Among these questions are key issues related to the extent to which the sympathetic nervous system should be inhibited or antagonized in CHF and, perhaps more importantly, the precise mechanism by which β-adrenoceptor antagonism exerts its beneficial actions in CHF. To this end, recent comparative studies of metoprolol and carvedilol (4), which show a relative benefit for the nonselective β-adrenoceptor antagonist, suggest that the β_2-adrenoceptor (and possibly α-adrenoceptors) could play a significant role in the pathogenesis of CHF.

Within the myocardium, the β_2-adrenoceptor is located at both sympathetic pre-synaptic and post-synaptic neuroeffector junctions, unlike the β_1-adrenoceptor, which is only post-junctional (5). In the post-synaptic location myocardial β-adrenoceptors are known to modulate heart rate and contractility, and in heart failure (HF) it has been shown that the β_1-adrenoceptor undergoes relatively greater down-regulation in comparison to the β_2-adrenoceptor (6). At the pre-synaptic site, experimental data exist to suggest that the β_2-adrenoceptor may facilitate the release of norepinephrine from nerve terminals, although this remains controversial. In opposition to this facilitatory role, the α_2-adrenoceptor has been shown to inhibit the neuronal release of norepinephrine (7,8). However, the precise functional status of

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Abbreviations and Acronyms
CHF = congestive heart failure
HF = heart failure
PCR = polymerase chain reaction

these pre-synaptic receptors remains uncertain in CHF. In addition to pre-synaptic modulation, the rate of catecholamine release from sympathetic nerve terminals is clearly under the influence of central control mechanisms, and the intrasynaptic concentration of norepinephrine is modulated by the neuronal norepinephrine transporter.

The clinical importance of understanding the interaction between cardiac sympathetic adrenergic drive and HF pathophysiology and outcome has been made even more important with the recent discovery of functional polymorphisms of the β_1-, β_2-, and α_2-adrenoceptors. In particular, it has been demonstrated that polymorphisms of the α_{2C}-adrenoceptor may be accompanied by a greater propensity to CHF (8,9), and that polymorphisms of the β_1- and β_2-adrenoceptors may alter the clinical profile of CHF or the clinical response to carvedilol (10,11). Despite these associative observations, it remains uncertain whether these polymorphisms truly alter the physiologic response of the myocardium to varying adrenergic drive. Furthermore, despite the potential for polymorphisms of the pre-synaptic α_2- and β_2-adrenoceptors to modify the release of norepinephrine from sympathetic nerve terminals, this has not been investigated in humans. Accordingly, in the present study we aimed to evaluate the effect of adrenergic receptor polymorphisms on the response of myocardium to measured levels of cardiac adrenergic drive, and secondly to evaluate whether polymorphisms of pre-synaptic adrenoceptors modified the rate of cardiac and systemic release of norepinephrine.

METHODS

Sixty patients undergoing right heart catheterization for evaluation of CHF status and/or heart transplant assessment participated in the study. The study group consisted of 56 men and four women age 58 ± 1 years (range 34 to 72 years). The etiology of CHF was ischemic cardiomyopathy in 48%, dilated cardiomyopathy in 47%, and other causes in the remaining 5%. Subjects were all of Caucasian background. The mean New York Heart Association functional class was 2.5 (range 2 to 4), the group mean LV ejection fraction was 19.9 ± 1.0% (mean ± SEM), and seven patients were in atrial fibrillation. The average duration of HF was 4.7 ± 1.3 years (range 2 to 25 years). All patients were treated with angiotensin-converting enzyme inhibitors and diuretics, 79% received digoxin, 37% were treated with carvedilol, and 23% received amiodarone. Medications were withheld only on the morning of the catheterization study to avoid potential hemodynamic instability. All patients gave written informed consent, and the study was approved by the Alfred Hospital Ethics Review Committee.

Hemodynamic evaluation and radiotracer measurement of sympathetic nervous activity. A radial arterial line and right internal jugular venous sheath were inserted under local anesthesia. This was followed by the measurement of resting central hemodynamics, using a pulmonary artery thermodilution catheter. A coronary sinus thermodilution catheter (Webster Laboratories, Baldwin Park, California) was subsequently positioned in the coronary sinus for blood sampling and blood flow measurement. Total systemic and cardiac norepinephrine spillover rates were determined according to well-described methods previously reported by our group (12). In brief, ^{3}H-labeled norepinephrine was continuously infused (0.5 to 1 μCi/min) via a peripheral vein to achieve steady-state plasma concentrations. The plasma norepinephrine concentration in arterial and coronary sinus blood was subsequently determined by high-performance liquid chromatography using electrochemical detection. The plasma-specific activity of ^{3}H-norepinephrine was determined by timed collection of the detector cell eluant and subsequent radioactivity counting by liquid scintillation spectroscopy.

Adrenoceptor genotyping. Genomic deoxyribonucleic acid isolated from peripheral blood was subjected to the polymerase chain reaction (PCR) for the determination of the β_1-adrenoceptor genotype at positions 49 and 389, the β_2-adrenoceptor genotype at positions 16 and 27, and to establish the presence or absence of the α_{2C}-adrenoceptor 322–325 deletion polymorphism according to previously published methods (11,13–15). In brief, for the determination of the β_1-adrenoceptor genotype at position 49 a 794-bp fragment (positions 1–794) of the β_1-adrenoceptor gene was amplified by PCR using the following primers: 5′-ATG GGC GGG GTG GTC-3′ (sense) and 5′-GAA ACG GCG CTC GCA GCT GTC G-3′ (antisense). The Ser49Gly polymorphism was determined according to the Eco0109I digestion pattern. For the β_1-adrenoceptor genotype at position 389 a 547-bp fragment of the β_1-adrenoceptor gene was amplified by PCR using the following primers: 5′-CAT CAT CGT CTT CAC GC-3′ (sense) and 5′-TGG GCT TCG AGT TCA CCT GC-3′ (antisense). The Gly389Arg polymorphism was determined according to the BcgI digestion pattern. To determine the β_2-adrenoceptor genotype a 308-bp fragment of the β_2-adrenoceptor gene was amplified by PCR using the following primers: 5′-GCC TCC TTG CTGGCA CCC CAT-3′ (sense) and 5′-GGA AGT CCA AAA CTC GCA CCA-3′ (antisense). The Arg16Gly polymorphism was determined according to the NcoI digestion pattern and the Gln27Glu polymorphism was evaluated according to the pattern that resulted from BbvI digestion. The α_{2C}-adrenoceptor genotype was determined according to the HaeIII digestion pattern of the PCR product generated using the primers 5′-CAT CTA CCG AGT GGC CAA GCT-3′ (sense) and 5′-AGC ACA AAG GTG AAG CGC TTC-3′ (antisense).