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,

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

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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 $19.9 \pm 1.0\%$ (mean $\pm$ 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).

Table 1. Group Hemodynamic Profile According to Adrenoceptor Genotype in Patients With Congestive Heart Failure

Adrenoceptor	Genotype	n	Heart Rate (beats/min)	MAP (mm Hg)	PCWP (mm Hg)	Cardiac Output (l/min)
Alpha-2C	wt/wt	52	77 ± 3	79 ± 1	17 ± 1	4.4 ± 0.2
	del/del	8	75 ± 3	86 ± 4	17 ± 3	4.7 ± 0.7
Beta-1 pos 49	Ser/Ser	42	75 ± 3	79 ± 2	17 ± 1	4.3 ± 0.2
	Ser/Gly	16	79 ± 5	82 ± 2	19 ± 3	4.0 ± 0.3
	Gly/Gly	2	83 ± 5	79 ± 5	11 ± 2	5.9 ± 1.5
Beta-1 pos 389	Arg/Arg	30	83 ± 5	83 ± 2	16 ± 2	4.4 ± 0.2
	Arg/Gly	22	72 ± 4	76 ± 2	17 ± 2	4.1 ± 0.3
	Gly/Gly	8	77 ± 4	80 ± 2	22 ± 2	4.1 ± 0.4
Beta-2 pos 16	Arg/Arg	15	72 ± 3	80 ± 3	15 ± 2	4.2 ± 0.3
	Arg/Gly	15	77 ± 3	80 ± 2	18 ± 3	4.6 ± 0.3
	Gly/Gly	30	83 ± 5	78 ± 3	18 ± 2	4.3 ± 0.3
Beta-2 pos 27	Gln/Gln	19	80 ± 4	81 ± 2	18 ± 2	4.5 ± 0.3
	Gln/Glu	29	80 ± 5	79 ± 2	17 ± 2	4.7 ± 0.4
	Glu/Glu	12	72 ± 3	79 ± 2	17 ± 2	4.1 ± 0.3

MAP = mean arterial pressure; PCWP = pulmonary capillary wedge pressure.

Statistical methods. Data are presented as mean values ± SEM. Between-group comparisons were performed by unpaired t test for two groups or one-way analysis of variance for comparison of three groups. Linear regression analysis was employed to investigate the relationship between independent variables. Analysis of covariance was employed to assess the influence of genotype on the relationship between two associated parameters. Chi-square analysis was performed to compare the distribution of categorical variables. SPSS version 11.0 (Chicago, Illinois) was used for statistical analysis. A p value <0.05 was considered statistically significant.

RESULTS

In the present study we observed a 83% frequency of the Ser[49] allele and a 68% frequency of the Arg[389] allele for the β_1-adrenoceptor gene, together with a 38% frequency for the Arg[16] allele and a 56% frequency of the Gln[27] allele for the β_2-adrenoceptor gene, consistent with previous studies (10,16). In respect of the α_{2C}-adrenoceptor, 13% of the study population was homozygous for the α_{2C} (322–325del) polymorphism, whereas no heterozygous individuals were detected. In the present study cohort, patient utilization of amiodarone and carvedilol therapy was uniform across all genotypes (data not shown).

Adrenoceptor genotype, hemodynamic and neurochemical profile. For the entire study cohort, the group mean data were: heart rate 77 ± 2 beats/min, mean arterial pressure 80 ± 1 mm Hg, mean pulmonary capillary wedge pressure 18 ± 1 mm Hg, mean pulmonary artery pressure 25 ± 1 mm Hg, cardiac output was 4.1 ± 0.1 l/min, plasma norepinephrine concentration 2.8 ± 0.2 pmol/ml, total systemic norepinephrine rate 5.3 ± 0.5 nmol/min, and cardiac norepinephrine spillover rate 288 ± 31 pmol/min. As shown in Table 1 the hemodynamic profile of our HF cohort was quite homogeneous across all of the adrenoceptor polymorphisms that were investigated. Our study had two major aims: first to examine the interaction between

cardiac sympathetic drive as assessed by the norepinephrine spillover method and myocardial response as reflected by the heart rate, and second to address the potential influence of polymorphic variations of the pre-synaptic α_{2C}- and β_2-adrenoceptors on the release of norepinephrine.

To investigate the theoretical ability of polymorphisms of the post-synaptic adrenoceptors (β_1 and β_2) to modify the heart rate response to neuronally released norepinephrine, we first investigated the relationship between heart rate and plasma catecholamines and catecholamine spillover rates. As shown in Figures 1A and 1B, modestly significant correlations were observed between heart rate and plasma norepinephrine and the total systemic norepinephrine spillover rate. Of note, the relationship between heart rate and total norepinephrine spillover was influenced by one outlying patient, and the relationship became nonsignificant when this data point was censored (r = 0.22, p = 0.10). As shown in Figure 1C, a robust relationship was observed between cardiac norepinephrine spillover rate and heart rate. To establish whether the relationship between cardiac norepinephrine spillover rate and heart rate was modified by polymorphisms of the β-adrenoceptors we performed an analysis of covariance according to differing genotypes for the varying genotypes. Under this analysis it was apparent the slope of the relationship between cardiac norepinephrine spillover rate and heart rate was significantly greater for patients with the β_2-adrenoceptor genotype Arg/Arg at position 16, compared with the combined Arg/Gly and Gly/Gly patient group (Fig. 2). The same relationship was also observed when patients treated with carvedilol (p = 0.04) or amiodarone (p = 0.03) were excluded from the analysis, and similarly when patients in atrial fibrillation were excluded (p = 0.04). A separate analysis of the three individual β_2- position 16 genotypes did not reveal evidence of a gene "dose-effect" relationship. In contrast, polymorphic variations of the β_2-adrenoceptor at position 27 (Fig. 2) or the β_1-adrenoceptor at positions 49
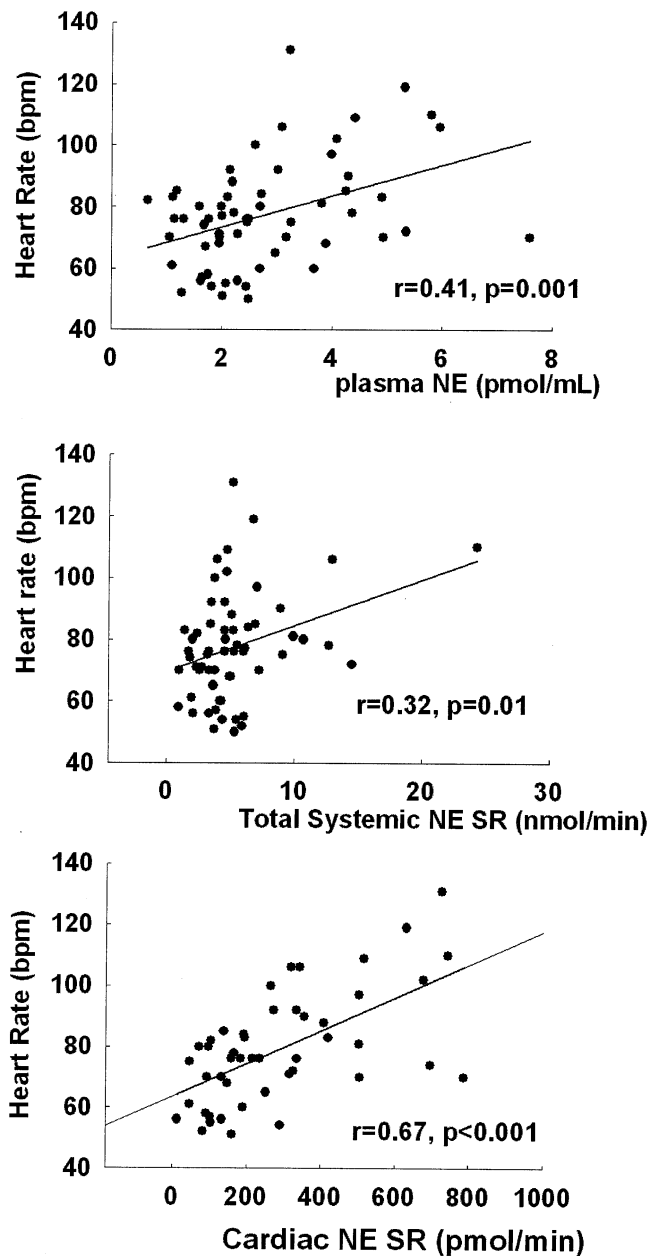
Figure 1. Scatterplots show the relationship between heart rate and plasma norepinephrine (NE) **(top panel)**, total systemic norepinephrine spillover rate (SR) **(middle panel)**, and cardiac norepinephrine spillover rate **(lower panel)**. The relationship between heart rate and total norepinephrine spillover **(middle panel)** is influenced by an outlying patient; when removed r = 0.22, p = 0.10. bpm = beats/min.

Figure 2. Scatterplots demonstrate the relationship between cardiac norepinephrine spillover (NE SR) and heart rate. The **top panel** indicates the relationship according to *β2*-adrenoceptor genotype at position 16 and the **lower panel** represents position 27. The **dashed line in the top panel** indicates the regression line for Arg/Arg individuals and the **solid line in the top panel** indicates the regression line for the combined Arg/Gly and Gly/Gly patients. The **slope of the line** is significantly different (p = 0.02). bpm = beats/min.

and 389 (Fig. 3) showed no statistically significant influence over the heart-rate-to-cardiac-norepinephrine spillover relationship. There was no evidence of a specific interactive haplotype effect for either the β_1- or β_2-adrenoceptor on the relationship between cardiac adrenergic drive and heart rate.

Our second aim was to establish whether polymorphisms of the pre-synaptic adrenoceptors had the capacity to influence the rate of norepinephrine release from sympathetic nerve terminals. We first sought to establish the key
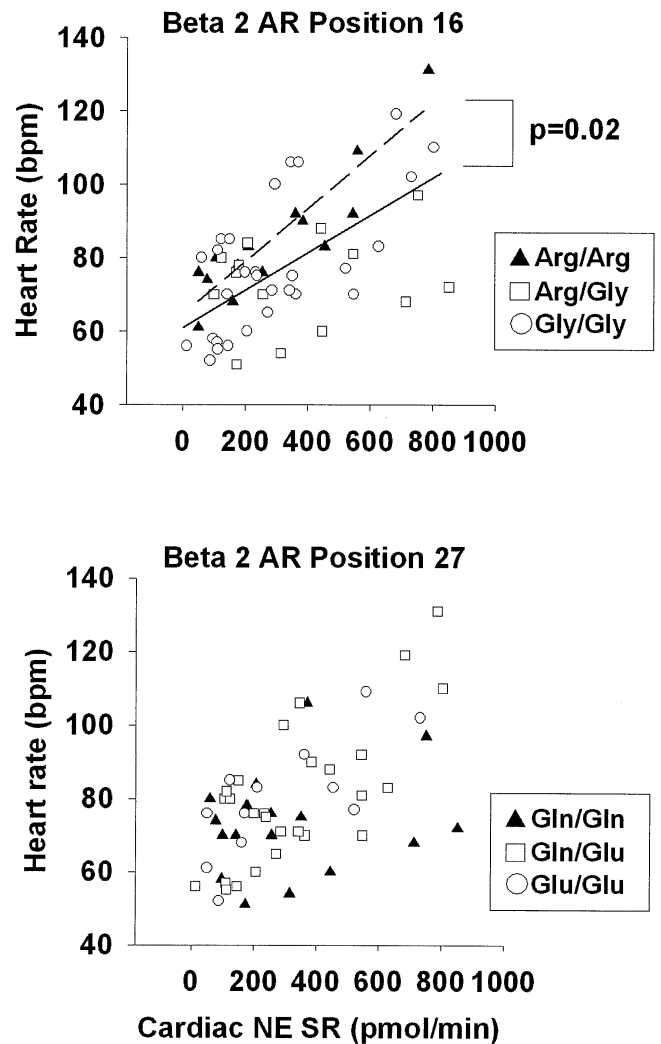
hemodynamic driving factors for activation of cardiac sympathetic nerve activity. As previously observed, the key hemodynamic factor appeared to be the pulmonary capillary wedge pressure (Fig. 4), compared with either the mean arterial pressure or cardiac output. As shown in Table 2, polymorphic variations of the α_2- or β_2-adrenoceptors showed no statistically significant influence over the rate of spillover of norepinephrine from sympathetic nerves in the heart specifically or more systemically as reflected by the total norepinephrine spillover rates. Analysis of covariance was also employed to avoid the confounding effects of variations in contributing hemodynamic factors such as the pulmonary capillary wedge pressure. Again, we were unable to establish an effect of pre-synaptic adrenergic receptor genotype on the rate of release of norepinephrine for either
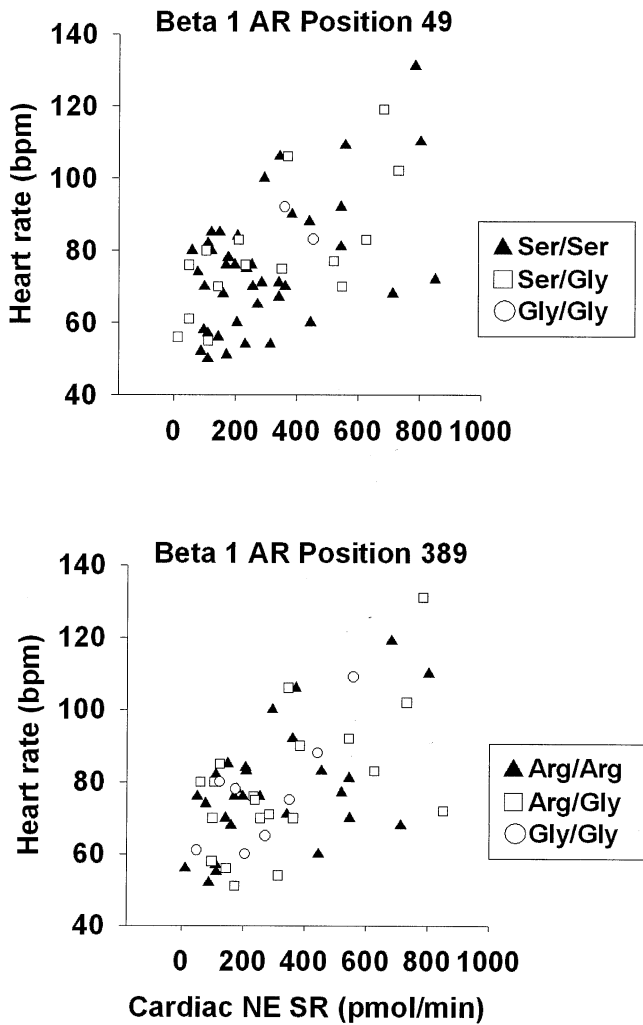
Figure 3. Scatterplots demonstrating the relationship between cardiac norepinephrine spillover (NE SR) and heart rate. The **top panel** indicates the relationship according to β_1-adrenoceptor genotype at position 49 and the **lower panel** represents position 389.

the β_2- or α_{2C}-adrenoceptors (Fig. 5). In conjunction, there was no evidence of a β_2-adrenoceptor haplotype effect on the cardiac norepinephrine spillover rate per se, or on the relationship between wedge pressure and norepinephrine spillover (data not shown).

DISCUSSION

It is now without doubt that the sympathetic nervous system plays a pivotal role in the progression of CHF. In particular, activation of the cardiac sympathetic nerves has been shown in CHF to be linked to adverse outcome (2) and occurs early in the development of left ventricular dysfunction (17). Clearly, the antagonism of excess exposure to norepinephrine within the failing heart as afforded by the use of β-adrenoceptor antagonists has contributed greatly to the management of the CHF patient. Recently a number of polymorphic variations of both α- and β-adrenoceptors have been identified (10). A number of these have been shown to have direct biochemical consequences, and some
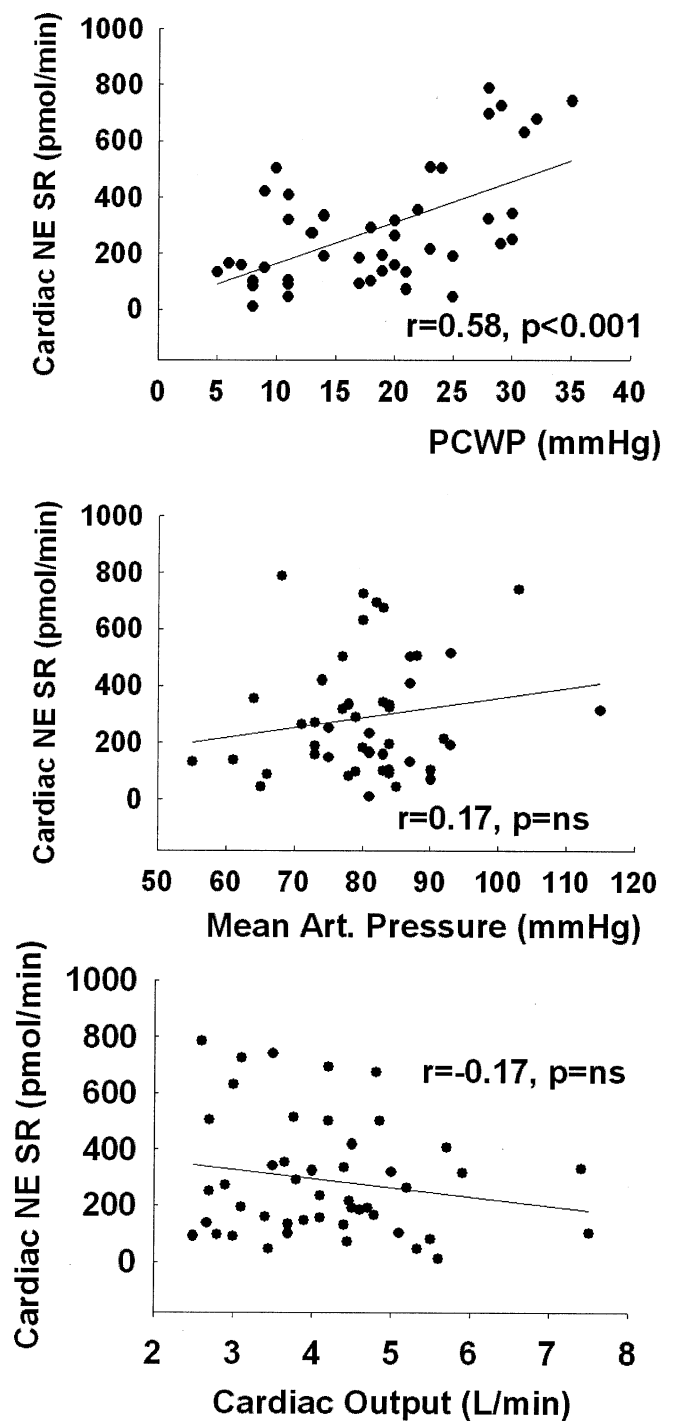
Figure 4. Scatterplots demonstrate the relationship between cardiac norepinephrine spillover rate (NE SR) and pulmonary capillary wedge pressure (PCWP) **(top panel)**, mean arterial (Art.) pressure **(middle panel)**, and cardiac output **(lower panel)**.

have been demonstrated clinically to be associated with pharmacological response to β-adrenoceptor agonists (18) and antagonists (11) or to be associated with the prevalence and clinical profile of HF (9,19).

Within the myocardium adrenergic receptors are located on multiple cell types, including cardiomyocytes and innervating sympathetic neurons. In the present study, we wished

Table 2. Norepinephrine Spillover According to Adrenoceptor Genotype in Patients With Congestive Heart Failure

Adrenoceptor	Genotype	Cardiac NE SR (pmol/min)	Systemic NE SR (nmol/min)
Alpha-2C	wt/wt	290 ± 82	5.4 ± 0.5
	del/del	295 ± 87	5.0 ± 1.3
Beta-2 pos 16	Arg/Arg	280 ± 59	5.3 ± 0.8
	Arg/Gly	344 ± 78	5.4 ± 0.8
	Gly/Gly	269 ± 40	5.4 ± 0.8
Beta-2 pos 27	Gln/Gln	277 ± 62	5.6 ± 0.8
	Gln/Glu	308 ± 42	4.1 ± 0.5
	Glu/Glu	261 ± 68	5.8 ± 0.9

NE = norepinephrine; SR = spillover rate.

specifically to assess the importance of functionally relevant polymorphisms of the β_1-, β_2-, and α_2-adrenoceptor in the context of the interaction between sympathetic nerves and the myocardium. We hypothesized that this interaction could, theoretically, be modified by genotype in two ways. First, polymorphisms of the β_1- or β_2-adrenergic receptors could influence the heart rate at a given level of adrenergic
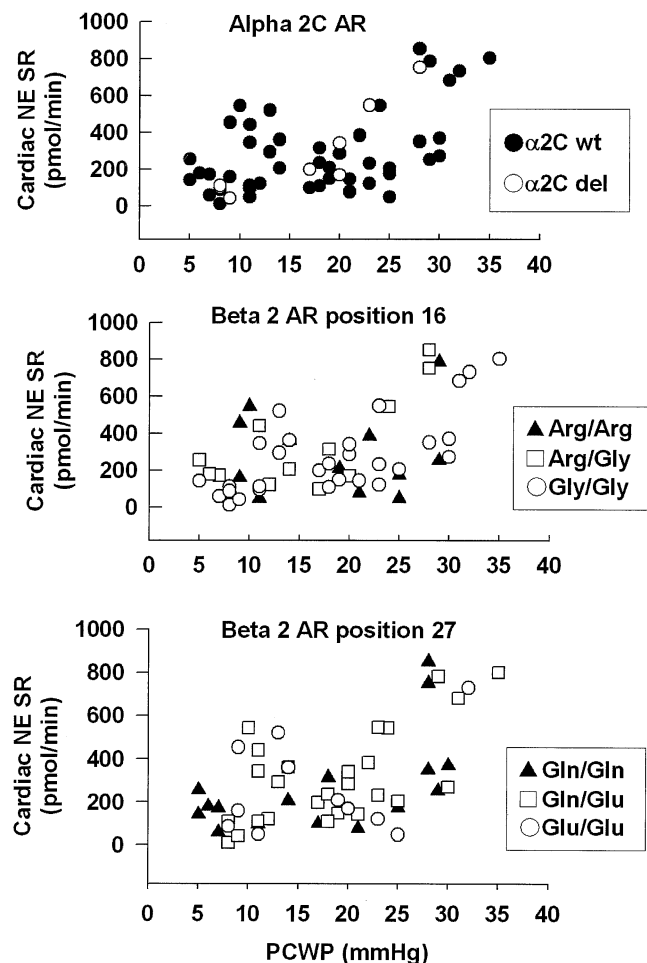

Figure 5. Scatterplot shows the relationship between pulmonary capillary wedge pressure (PCWP) and cardiac norepinephrine spillover rate (NE SR) according to α_{2C}-adrenoceptor genotype **(top panel)**, β_2-adrenoceptor genotype at position 16 **(middle panel)**, and β_2-adrenoceptor genotype at position 27 **(lower panel)**.

drive, and second, polymorphisms of adrenergic receptors located pre-synaptically could modify the release of norepinephrine for a given level of cardiac sympathetic nerve firing.

In the context of our first question, we demonstrated a strong relationship between the prevailing level of cardiac adrenergic drive as reflected by the cardiac norepinephrine spillover rate and heart rate in patients with HF. When we examined the effect of β_1- or β_2-adrenoceptor genotype as potential modifiers, we showed, for the first time, that patients who were homozygous for the Arg residue at position 16 of the β_2-adrenoceptor demonstrated a significantly greater heart rate for a given level of adrenergic input. These data are consistent with the known biochemical profile of polymorphisms at this locus. Specifically, the Gly16 polymorphism is associated with enhanced agonist-promoted downregulation in contrast to Arg16 (20). At a clinical level, few studies have examined the interaction between stimuli that alter cardiac adrenergic drive and hemodynamic response in the context of adrenergic receptor polymorphisms, and none has directly measured cardiac adrenergic drive as in the present study. In a previous study Wagoner et al. (19) showed that the exercise response in patients with CHF who had the β_2-adrenoceptor Gly16 polymorphism had reduced exercise capacity, although in their study heart rate was no different at rest or during exercise. Although in the present study we cannot comment about the role of polymorphisms of the β-adrenoceptors in the context of HF progression for a given level of adrenergic drive, it is also noteworthy that unlike the β_1-adrenoceptor, it has been demonstrated that the β_2-adrenoceptor does not undergo downregulation in HF (6). As such, the role assumed by polymorphisms of the myocardial β_2-adrenoceptor may assume even greater relevance in the pathophysiology of CHF.

Our study is also the first to examine the relationship between α_{2C}- and β_2-adrenoceptor genotype and catecholamine release in human congestive HF. We quantitated the rate of release of norepinephrine from cardiac sympathetic nerve terminals using the norepinephrine spillover technique, as previously characterized by our group. This study was predicated on recent studies that have drawn an association between the presence of polymorphic variations in the α_{2C}- and β_2-adrenoceptor and the prevalence or severity of CHF (9,19) and the theoretical influence of these polymorphisms on the pre-synaptic control of neurotransmitter release. Our study does not provide support for the evidence that genetic polymorphisms of pre-synaptic adrenoceptors play a role in the pathophysiology of CHF, but by virtue of their apparent lack of effect on norepinephrine release from sympathetic nerves. In this study we also attempted to correct for possible variations in the levels of major hemodynamic stimuli that might alter the rate of cardiac sympathetic nerve firing.

The α_2-adrenoceptor has long been known to regulate the rate of release of norepinephrine from sympathetic nerve

terminals, both in vitro and in vivo (8,21). Several polymorphisms of the various α_2-adrenoceptors have been identified, but most recently the α_{2C}-del 322–325 has been ascribed particular importance after the demonstration of an association between its presence and an increased incidence of HF (9). In the present study we could not demonstrate any apparent relationship between the presence or absence of this mutation and the rate of catecholamine release from sympathetic nerves. Although functional data suggest this mutation is associated with reduction function (22), its influence on catecholamine release has not been previously tested in vivo or in vitro. In the present study, the apparent lack of influence of the α_{2C}-adrenoceptor polymorphism on the rate of release of norepinephrine from the heart could readily be explained by a downregulation of the pre-synaptic α_2-adrenoceptor in CHF, as we have previously shown (21).

Pre-synaptic β_2-adrenoceptors have been proposed to play a facilitatory role in the release of norepinephrine from sympathetic nerve terminals in the heart, although this remains controversial (23,24). As with the α_{2C}-adrenoceptor, several polymorphisms of the β_2-adrenoceptor that are associated with functional consequences have been described. In particular, the Gly16 and Gln27 polymorphisms have been shown to occur in a significant proportion of the population and are associated with increased agonist-induced downregulation (10). In the present study we did not observe a relationship between the presence of either polymorphism and an altered norepinephrine spillover rate, suggesting that either the β_2-adrenoceptor itself does not play a functional role in regulating norepinephrine release from sympathetic nerves in CHF, or that the documented biochemical effects of the polymorphisms are of no physiologic significance with reference to the modulation of norepinephrine release. In support of the former possibility, Lameris et al. (24) recently showed that an intracoronary infusion of epinephrine was without effect on the myocardial interstitial norepinephrine concentration as assessed by microdialysis.

As such, given our current data, it is more likely that studies that have associated β_2-adrenoceptor genotype and measures of functional capacity and possibly outcome, relate more to the level of expression and function of myocardial β_2-adrenoceptors. Consistent with this hypothesis, we have recently shown that the influence of carvedilol on left ventricular function in patients with CHF was related to the β_2-adrenoceptor genotype at position 27 (11), whereas it does not alter the release of norepinephrine from the failing heart.

Study limitations. Our study is the first to specifically examine the interaction between adrenergic drive to the failing heart and the associated cardiac response in the context of polymorphic variations in adrenergic receptors. Our study was of an invasive nature, and as such, the number of subjects included was of a modest size. Accordingly, it is conceivable that our study was not sufficiently powered to detect the potential influences of certain of the genotypes or their interactions with relevant drugs, including carvedilol and amiodarone. Digoxin use was also prevalent, although we have previously shown that digoxin does not appear to modify cardiac adrenergic drive (25). Further, given the cohort size, it was unlikely that our study would have detected any significant interactions between genotypes. Moreover, in contrast to the study by Small et al. (9), our study population did not include an African American cohort, thus limiting the number of individuals with the α_{2C} deletion polymorphism, thereby limiting further haplotype analysis. Larger studies would be required to meaningfully investigate haplotype analysis of this nature.

Conclusions. Our study has, for the first time, sought to investigate the basis for previously reported associations between polymorphic variations in adrenergic receptors and the frequency or severity of HF. Specifically, we employed detailed radiotracer methodology to investigate the impact of α_{2C}- and β_2-adrenoceptor genotype on the relationship between a given level of cardiac adrenergic drive and the myocardial response to that stimulus, and to investigate the influence of polymorphisms of pre-synaptic adrenergic receptors on the rate of cardiac and total systemic norepinephrine spillover in the setting of human HF. Our studies indicate that the myocardium response to a given level of adrenergic drive can be modified by adrenergic receptor genotype, whereas polymorphic differences in pre-synaptic adrenoceptor genotype do not modify the rate of norepinephrine release from sympathetic nerves.

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REFERENCES

1. Cohn J, Levine T, Olivari M, et al. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. N Engl J Med 1984;311:819–23.
2. Kaye DM, Lefkovits J, Jennings G, Bergin P, Broughton A, Esler MD. Adverse consequences of increased cardiac sympathetic activity in the failing human heart. J Am Coll Cardiol 1995;26:1257–63.
3. Bristow M. Antiadrenergic therapy of chronic heart failure: surprises and new opportunities. Circulation 2003;107:1100–2.
4. Poole-Wilson PA, Swedberg K, Cleland JG, et al. Comparison of carvedilol and metoprolol on clinical outcomes in patients with chronic heart failure in the Carvedilol Or Metoprolol European Trial (COMET): randomised controlled trial. Lancet 2003;362:7–13.
5. Xiao RP, Cheng H, Zhou YY, Kuschel M, Lakatta EG. Recent advances in cardiac $\beta2$-adrenergic signal transduction. Circ Res 1999;85:1092–100.
6. Bristow MR, Ginsberg R, Umans V, et al. $\beta1$- and $\beta2$-adrenergic receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective $\beta1$-receptor down-regulation in heart failure. Circ Res 1986;59:297–309.
7. Majewski H. Modulation of norepinephrine release through activation of presynaptic β-adrenoceptors. J Auton Pharm 1983;3:47–60.
8. Brede M, Wiesmann F, Jahns R, et al. Feedback inhibition of catecholamine release by two different alpha2-adrenoceptor subtypes prevents progression of heart failure. Circulation 2002;106:2491–6.
9. Small KM, Wagoner LE, Levin AM, Kardia SL, Liggett SB. Synergistic polymorphisms of $\beta1$- and $\alpha2C$-adrenergic receptors and the risk of congestive heart failure. N Engl J Med 2002;347:1135–42.

10. Small KM, McGraw DW, Liggett SB. Pharmacology and physiology of human adrenergic receptor polymorphisms. Annu Rev Pharmacol Toxicol 2003;43:381–411.
11. Kaye DM, Smirk B, Williams C, Jennings G, Esler M, Holst D. Beta-adrenoceptor genotype influences the response to carvedilol in patients with congestive heart failure. Pharmacogenetics 2003;13:379–82.
12. Esler M, Jennings G, Lambert G, Meredith I, Horne M, Eisenhofer G. Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. Physiol Rev 1990;70:963–85.
13. Borjesson M, Magnusson Y, Hjalmarson A, Andersson B. A novel polymorphism in the gene coding for the β1-adrenergic receptor associated with survival in patients with heart failure. Eur Heart J 2000;21:1853–8.
14. O'Shaughnessy KM, Fu B, Dickerson C, Thurston D, Brown MJ. The gain of function variant of the β1-adrenoceptor does not influence blood pressure or heart rate response to β-blockade in hypertensive subjects. Clin Sci 2000;99:233–8.
15. Tsai S-J, Wang Y-C, Hong C-J. Norepinephrine transporter and $\alpha2_C$ allelic variants and personality factors. Am J Med Genetics 2002;114:649–51.
16. Liggett SB, Wagoner LE, Craft LL, et al. The Ile164 β2-adrenergic receptor polymorphism adversely affects the outcome of congestive heart failure. J Clin Invest 1998;102:1534–9.
17. Rundqvist B, Elam M, Bergmann-Sverrisdottir Y, Eisenhofer G, Friberg P. Increased cardiac adrenergic drive precedes generalized sympathetic activation in human heart failure. Circulation 1997;95:169–75.
18. Dishy V, Sofowora GG, Xie HG, et al. The effect of common polymorphisms of the β2-adrenergic receptor on agonist-mediated vascular desensitization. N Engl J Med 2001;345:1030–5.
19. Wagoner LE, Craft LL, Singh B, et al. Polymorphisms of the β2-adrenergic receptor determine exercise capacity in patients with heart failure. Circ Res 2000;86:834–40.
20. Green SA, Turki J, Innis M, Liggett SB. Amino-terminal polymorphisms of the human beta 2-adrenergic receptor impart distinct agonist-promoted regulatory properties. Biochemistry 1994;33:9414–9.
21. Aggarwal A, Esler MD, Socratous F, Kaye DM. Evidence for functional presynaptic alpha-2 adrenoceptors and their down-regulation in human heart failure. J Am Coll Cardiol 2001;37:1246–51.
22. Small KM, Forbes SL, Rahman FF, Bridges KM, Liggett SB. A four amino acid deletion polymorphism in the third intracellular loop of the human α2C-adrenergic receptor confers impaired coupling to multiple effectors. J Biol Chem 2000;275:23059–64.
23. Newton GE, Azevedo ER, Parker JD. Inotropic and sympathetic responses to the intracoronary infusion of a β2-receptor agonist: a human in vivo study. Circulation 1999;99:2402–7.
24. Lameris TW, de Zeeuw S, Duncker DJ, et al. Epinephrine in the heart: uptake and release, but no facilitation of norepinephrine release. Circulation 2002;106:860–5.
25. Kaye DM, Lambert GW, Lefkovits J, Morris M, Jennings G, Esler MD. Neurochemical evidence of cardiac sympathetic activation and increased central nervous system norepinephrine turnover in severe congestive heart failure. J Am Coll Cardiol 1994;23:570–8.