Conservation of the Cardiostimulant Effects of \((-\))-Norepinephrine Across Ser49Gly and Gly389Arg Beta1-Adrenergic Receptor Polymorphisms in Human Right Atrium In Vitro

Peter Molenaar, PhD,* Glenn Rabnott, BSc (Hons),† Ian Yang, MBBS, FRACP,† Kwun M. Fong, MBBS, PhD, FRACP,† Santiyagu M. Savarimuthu, MSc,† Li Li, MD,* Malcolm J. West, MBBS, PhD, FRACP,* Fraser D. Russell, PhD*

Queensland, Australia

OBJECTIVES

The goal of this study was to determine whether the cardiostimulant effects of the endogenous beta1-adrenergic receptor (AR) agonist, \((-\))-norepinephrine are modified by polymorphic (Serine49Glycine [Ser49Gly], Glycine389Arginine [Gly389Arg]) variants of beta1-ARs in the nonfailing adult human heart.

BACKGROUND

Human heart beta1-ARs perform a crucial role in mediating the cardiostimulant effects of \((-\))-norepinephrine. An understanding of the significance of Ser49Gly and Gly389Arg polymorphisms in the human heart is beginning to emerge, but not as yet in adult patients who have coronary artery disease (CAD).

METHODS

The potency and maximal effects of \((-\))-norepinephrine at beta1-ARs (in the presence of beta2-AR blockade with 50 nM ICI 118,551 [erythro-DL-1(7-methylindan-4-ylxy)-3-isopropylamino-butan-2-ol]) for changes in contractile force and shortening of contractile cycle duration were determined in human right atrium in vitro from 87 patients undergoing coronary artery bypass grafting who were taking beta-blockers before surgery. A smaller sample of patients (n = 20) not taking beta-blockers was also investigated. Genotyping for two beta1-AR polymorphisms (Ser49Gly and Gly389Arg) was determined from a sample of blood taken at the time of surgery.

RESULTS

\((-\))-Norepinephrine caused concentration-dependent increases in contractile force and reductions in time to reach peak force and time to reach 50% relaxation. There were no differences in the potency or maximal effects of \((-\))-norepinephrine at beta1-ARs (in the presence of beta2-AR blockade with 50 nM ICI 118,551) between patients with different Ser49Gly and Gly389Arg polymorphisms.

CONCLUSIONS

The cardiostimulant effects of \((-\))-norepinephrine at beta1-ARs were conserved across Ser49Gly and Gly389Arg polymorphisms in the right atrium of nonfailing hearts from patients with CAD managed with or without beta-blockers. (J Am Coll Cardiol 2002;40:1275–82) © 2002 by the American College of Cardiology Foundation

The human beta1-adrenergic receptor (AR) performs a crucial role in mediating the cardiostimulant effects of \((-\))-norepinephrine in the human heart. Within the coding block of the beta1-AR, 17 nucleotide substitutions have been identified, resulting in seven amino-acid substitutions, with two occurring in the extracellular amino-terminal and the remainder in the intracellular carboxyl-terminal (1–5). This raises several important questions concerning whether beta1-AR polymorphisms contribute to the etiology of disease and/or have a determining affect on patient prognosis. Intrinsically related to this is the question of whether beta1-AR polymorphisms interact differently to \((-\))-norepinephrine, other beta1-AR stimulants, and beta1-blockers. Results of studies addressing these questions are emerging.

The search for associations between beta1-AR polymorphisms and disease have thus far extended to heart failure (HF) and hypertension. An association between idiopathic dilated cardiomyopathy in a small population (37 patients) and the amino acid substitution of serine (Ser) (wild-type) by glycine (Gly) (mutation) at amino acid position 49 in the extracellular amino-terminal of the beta1-AR was observed (5). In a study of a larger population of patients with congestive HF (184 patients) with age-matched controls (77 patients), Börjesson et al. (4) showed no difference in mutation (Ser49Gly) frequency, but they observed improved survival in patients with the Gly49 mutation. Furthermore, the difference was more pronounced in patients receiving beta-blocker treatment.

The CARDIGENE population in France, comprising 426 patients with idiopathic dilated cardiomyopathy, was investigated for an association with the substitution of arginine (Arg) (mutant) for Gly (wild-type) at amino acid position 389 in the cytoplasmic tail of the beta1-AR, but none was observed (3). However, at a functional level, it was
observed that patients with HF (left ventricular [LV] ejection fraction 26 ± 1%) homozygous for Gly389-beta1-AR were associated with depressed exercise capacity compared with their homozygous Arg389-beta1-AR counterparts (6). An association between the homozygous Arg389 genotype and increased diastolic blood pressure and heart rate (HR) was observed in a population of hypertensive patients (7). The authors speculated that hypertension could be caused by increased activity of the Arg389 variant and consequent elevated cardiac output (7). Both studies are in line with original work at the molecular level, where it was shown that clonal cell lines expressing only the Arg389 variant had both greater basal and (-)isoproterenol-stimulated adenyl cyclase activity with tighter Gs-alpha-protein coupling, than cells expressing only Gly389 (2).

These studies raise the fundamental question of whether the potency and effectiveness of (-)-norepinephrine at human heart beta1-ARs is influenced by polymorphic (Ser49Gly, Gly389Arg) variations. In young healthy adults, this does not appear to be the case where exercise-mediated increases in HR caused by cardiac stimulation of beta1-ARs by neuronally released (-)-noradrenaline were not different for homozygous Gly389 and Arg389 groups (8,9), while Ser49Gly polymorphisms have not been investigated yet. In this study we investigated the cardiomodulator effects of (-)-norepinephrine at both Ser49Gly and Gly389Arg polymorphisms directly on the human heart (in vitro) from patients with coronary artery disease (CAD). We surveyed 531 patients who had undergone coronary artery bypass graft (CABG) surgery at the Prince Charles Hospital and ascertained that 78% were treated with a beta-blocker before surgery. Perioperatively, some patients were also prescribed infusions of dopamine and/or (-)-norepinephrine to maintain cardiac output and perfusion pressure. We considered that it was important to determine the significance of beta1-AR polymorphism in this group of patients, both in terms of the immediate effects of cardiomodulators and of long-term administration of beta1-blockers.

This study showed a uniformity of agonist potencies and effects of (-)-norepinephrine at polymorphic (Ser49Gly and Gly389Arg) beta1-ARs in the human right atrium from patients treated with atenolol or metoprolol undergoing CABG surgery. This observation is consistent with the effects of endogenous (-)-norepinephrine in healthy young adults who were not treated with beta-blockers (8,9).

A preliminary account of this work was presented at the 40th National Scientific Conference of the Australian Society for Medical Research (10).

### METHODS

This study was approved by The Prince Charles Hospital ethics committee (EC9876).

**Patients.** Human right atrial appendages and arterial blood samples were obtained from patients undergoing CABG surgery at The Prince Charles Hospital who were taking beta-blockers and who had given prior informed written consent. A description of patient characteristics according to genotype groupings is given in Table 1. Briefly, a comparison between groups indicated that these were made up of patients of similar age and body mass index, took similar classes of medications including doses of beta-blocker (atenolol/metoprolol), and had similar hemodynamic measurements (LV ejection fraction, LV end-systolic and -diastolic pressures). In addition, the patients making up these groups had a similar distribution of risk factors (hypercholesterolemia, hypertension, diabetes mellitus, obesity, and history of smoking).

A smaller sample of patients (20 patients) who were not taking beta-blockers before surgery were also investigated for possible differences in the effects of (-)-norepinephrine that could be attributed to beta1-AR polymorphism. In comparison with the overall cohort of beta-blocked patients, non-beta-blocked patients were older (68 ± 9 years [SD]) but had a similar LV ejection fraction (64 ± 3%). One reason for the exclusion of beta-blockers for the overall management of these patients included the existence of airways disease (eight patients). These patients received one or all of inhalational beta-receptor agonists, corticosteroids, and muscarinic receptor antagonists.

**Preparation of tissues.** After surgical removal, atrial tissues were placed immediately into ice-cold pre-oxygenated modified Krebs solution (containing mM: Na⁺ 125, K⁺ 5, Ca²⁺ 2.25, Mg²⁺ 0.5, Cl⁻ 98.5, SO₄²⁻ 0.5, HCO₃⁻ 32, HPO₄²⁻ 1, ethylenediamine-tetraacetic acid 0.04) and transported to the laboratory (within 5 min of surgical removal), where atrial strips containing intact trabeculae were dissected under continuous oxygenation. Tissues were mounted onto electrode blocks in 50-mL tissue baths and electrically stimulated to contract at 60 beats/min. Tissues were incubated with 5 μM phenylephrine to block alpha-ARs and neuronal and extraneuronal uptake of catecholamines and 50 nM ICI 118,551 to block beta2-ARs (11).

**Catecholamine concentration-effect curves.** Experiments were carried out only in tissues with a basal contractile force >1 mN. Both current and past experience from this laboratory have revealed that human right atrial tissues with

<table>
<thead>
<tr>
<th>Abbreviations and Acronyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR</td>
</tr>
<tr>
<td>Arg</td>
</tr>
<tr>
<td>bp</td>
</tr>
<tr>
<td>CABG</td>
</tr>
<tr>
<td>CAD</td>
</tr>
<tr>
<td>DMSO</td>
</tr>
<tr>
<td>DNA</td>
</tr>
<tr>
<td>Gly</td>
</tr>
<tr>
<td>HF</td>
</tr>
<tr>
<td>HR</td>
</tr>
<tr>
<td>LV</td>
</tr>
<tr>
<td>PCR</td>
</tr>
<tr>
<td>Ser</td>
</tr>
</tbody>
</table>
basal contractile force <1 mN often exhibit poor developed force and variable potencies to a variety of added stimulants. In practice, few (approximately 1% to 2%) were excluded on this basis.

Cumulative concentration-effect curves were established to (−)-norepinephrine in the presence of ICI 118,551 (erythyl- DL-1-[2-methylindan-4-oxyloxy]-3-isopropylaminobutan-2-ol) (selective β1-AR stimulation) by the sequential addition of agonist to the tissue bath followed by a single concentration (200 μM) of (−)-isoproterenol to obtain a maximal effect through stimulation of both β1- and β2-ARs (11). Experiments were concluded by raising the Ca2+ concentration to 9.25 mM.

Recordings of contractile force and first derivative were made on a Watanabe 12-channel recorder (Graphtec Corp., Yokohama, Japan). Time to reach peak force (determined as the time in which the first derivative was positive) and time to reach 50% relaxation were made from fast-speed (100 mm s⁻¹) recordings. Fast-speed recordings were obtained from 77 of 87 patients.

**Genotyping for Ser49Gly- and Gly389Arg- β1-AR polymorphisms.** Genotyping for β1-AR polymorphisms was carried out after completion (including analysis) of contractile studies and by different investigators, without knowledge of the results of the contractile studies. Genomic deoxyribonucleic acid (DNA) was isolated from peripheral blood leukocytes using a standard salt extraction method (12).

The identity of the amino acid at position 49 was determined by customizing the reaction described previously by Maqbool et al. (1). The identity of the amino acid at position 389 was determined by using the method described by Mason et al. (2) with minor modifications. The basic polymerase chain reaction (PCR) mixture was the same for each loci, with the exception of the concentration of dimethyl sulfoxide (DMSO) and the primer pair used. For each PCR, 10 ng of DNA was added to the reaction mixture with a final volume of 20 μl containing 10% RedTaq PCR reaction buffer, 0.2 mM of each deoxynucleotide triphosphate, 0.25 μM of each primer, and 0.5 U RedTaq Polymerase (RedTaq products from Sigma-Aldrich Pty Ltd., Castle Hill, Australia). For position 49 genotyping, 5% DMSO was used, and the primers were 5’-CCGGGCCCTCCGAGGTTCC-3’ (sense) and 5’-GGCGAGGTAGC-3’ (antisense). Cycling conditions were 94°C for 3 min; 94°C for 30 s, 65°C for 30 s, 72°C for 45 s for 40 cycles, and 72°C for 7 min. Amplification produced a 564 base pair (bp) product. For position 389 genotyping, 7.5% DMSO was used, and the primers were 5’-CCGCTCTCTCGTCTCTCCAAGTG-
Human Heart Beta1-AR Polymorphisms

![Figure 1](image_url)

Figure 1. Genotyping of the beta1-adrenergic receptor at nucleotide 145 (Ser49Gly) (A) and 1165 (Gly389Arg) (B) in patients undergoing coronary artery bypass grafting from which the right atrium was obtained and used to assess the contractile effects of (-)-norepinephrine at polymorphic beta1-adrenergic receptors. The figure shows polymerase chain reaction fragments of the predicted length after endonuclease digestion to discriminate between patients homozygous for Ser49, Gly49, or heterozygous for Ser49Gly (A) or homozygous for Gly389, Arg389, or heterozygous for Gly389Arg (B). Arg = arginine; bp = base pair; Gly = glycine; Ser = serine.

3’ (sense) and 5’-TGGGCTTCGAGTTCCACC-TGCTATC-3’ (antisense). Cycling conditions were 94°C for 3 min; 94°C for 30 s, 63°C touchdown to 58°C for 30 s, 72°C for 45 s for 5 cycles; 94°C for 30 s, 58°C for 30 s, 72°C for 45 s for 35 cycles, and 72°C for 7 min. The resulting product was 463 bp. Restriction digestion was used to distinguish between the alleles at both positions. At position 49, the Gly49 allele contained an Eco 0109 I restriction site resulting in products of 463 bp and 219 bp while the Ser49 allele remained uncleaved. The restriction enzyme BsmF1 cleaved at position 389 if the Gly389 allele was present, resulting in products of 352 and 111 bp but remained uncleaved if the Arg389 allele was present. Digestion products were run on a 2% agarose gel containing ethidium bromide and visualized using a Molecular Imager FX (Bio-Rad Laboratories Pty Ltd., Hercules, California).

The results of restriction enzyme genotyping were verified by direct sequencing. A DNA template was prepared for sequencing by PCR of the fragment purified by the Promega Wizard kit. Sequencing was performed using the Applied Biosystems PRISM system using an automated sequencer (QIMR Sequencing Facility, Brisbane, Australia).

**Statistics and calculations.** Data are expressed as mean ± SE or SD where indicated. Comparisons between the midpoints of cumulative concentration–effect curves (−logEC_{50} values) for (−)-norepinephrine determined from Ser49Ser and Ser49Gly groups were carried out using the Student t test. Comparisons between −logEC_{50} values for (−)-norepinephrine for Gly389Gly, Gly389Arg, and Arg389Arg groups were carried out using a one-way analysis of variance. The Student t test (Ser49Ser, Ser49Gly) and one-way analysis of variance statistical tests were used to compare the maximal effects of (−)-norepinephrine (relative to (−)-isoproterenol, I.A. (maximal effect of (−)-

**RESULTS**

**Patient population and genotyping.** Eighty-seven patients taking beta-blockers were recruited (Table 1) and genotyped for Ser49Gly- and Gly389Arg-beta1-AR polymorphisms (Fig. 1). Allele frequencies of 0.87 and 0.13 for Ser49 and Gly49, respectively, and 0.29 and 0.71 for Gly389 and Arg389, respectively, were observed (Table 1). Positive inotropic and lusitropic effects of (−)-norepinephrine at Ser49Gly- and Gly389Arg-beta1-AR polymorphisms in right atrium from patients taking beta1-blockers before surgery. Right atrial trabeculae were characterized by strong basal contractile forces that did not differ among the beta1-AR polymorphic groups (Fig. 2, p > 0.05). Stimulation of beta1-ARs with (−)-norepinephrine caused concentration-dependent increases in contractile force and shortening of the time to reach peak force and time to reach 50% relaxation (Fig. 2, Table 2). There were no differences in potency or maximal effects of (−)-norepinephrine at Ser49Gly or Gly389Arg polymorphisms (p > 0.05). Further analysis also revealed no differences when Ser49Gly polymorphisms were considered in patients homozygous for Arg389 or Gly389Arg polymorphisms were considered in patients homozygous for Ser49 (p > 0.05, data not shown).
Figure 2. Concentration-dependent effects of (−)-norepinephrine for changes in contractile force, time to reach peak force, and time to reach 50% relaxation (t50) through activation of Ser49Gly- and Gly389Arg-polymorphisms of the beta1-adrenergic receptor in human right atrium. Contractile force is expressed in terms of absolute force (mN). Experiments were completed by incubation of tissues with 200 μM (−)-isoproterenol to maximally stimulate both beta1- and beta2-adrenergic receptors followed by raising the concentration of Ca2+ to 9.25 mM. Values shown are mean ± SEM, where SEM values were determined from each specific log (−)-norepinephrine concentration. Errors not shown when smaller than symbol. Values in parenthesis show (tissue number/patient number). Arg = arginine; Gly = glycine; Ser = serine.
Does long-term administration of β1 blockers nullify possible differences between β1-AR polymorphisms? To determine whether long-term administration of β1-AR blockers nullifies differential (−)-norepinephrine effects at β1-AR polymorphisms, we investigated the effects of (−)-norepinephrine in the right atrium of patients who did not receive β-blockers before surgery. In a small group of patients (n = 20), a small (2.4-fold) reduction in the potency of (−)-norepinephrine for positive inotropic effects was observed compared with patients receiving β1-blockers before surgery. However, there were no differences in the potency or maximal effects of (−)-norepinephrine between homozygous Ser49 patients and those carrying the Gly49 variant, and there were also no differences in potency between homozygous Arg389 patients and those carrying the Gly389 variant (p > 0.05, Table 3).

### DISCUSSION

(−)-Norepinephrine has similar potencies and efficacies at two β1-AR polymorphisms, Ser49Gly and Gly389Arg for changes in contractile force and shortening of contractile cycle in the right atrium of non-failing hearts from patients with CAD undergoing CABG surgery.

**β1-AR polymorphisms and function in the human heart.** Our study was motivated by the need to determine the clinical significance of β1-AR polymorphisms directly in the human heart. In this study we used a carefully defined population comprised of patients undergoing CABG surgery. In this group of patients, therapeutic strategies often utilize the β1-AR. β1-blockers are commonly used to manage patients with CAD, and if they proceed to CABG surgery, there is the possibility they may also receive inotropic support with dopamine and/or (−)-norepinephrine peripherally. For this group of patients, two β1-AR polymorphisms, Ser49Gly and Gly389Arg, did not differentially affect (−)-norepinephrine effects at the β1-AR.

The same conclusion for Gly389Arg was also reached in two recent in vivo studies of exercise-induced (endogenous (−)-norepinephrine), cardiac effects in young healthy individuals (8,9). The human right atrial appendage from which we obtained right atrial muscle could also be considered to be representative of the human right atrial myocardium.

### Table 2. Inotropic and Lusitropic Potencies and Intrinsic Activities of (−)-Norepinephrine at Polymorphic β1-Adrenergic Receptors in Right Atrial Trabeculae From Patients Having Long-Term Treatment With Beta-Blockers

<table>
<thead>
<tr>
<th>Effect</th>
<th>Genotype</th>
<th>−LogEC50</th>
<th>IA</th>
<th>%Ca2⁺</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Force</td>
<td>Ser49Ser</td>
<td>7.4 ± 0.1</td>
<td>0.99 ± 0.01</td>
<td>79.9 ± 2.3</td>
<td>102/67</td>
</tr>
<tr>
<td></td>
<td>Ser49Gly</td>
<td>7.4 ± 0.1</td>
<td>1.01 ± 0.02</td>
<td>83.3 ± 2.9</td>
<td>28/18</td>
</tr>
<tr>
<td></td>
<td>Gly49Gly</td>
<td>7.5</td>
<td>1.02</td>
<td>69.2</td>
<td>2/2</td>
</tr>
<tr>
<td>t50</td>
<td>Ser49Ser</td>
<td>7.5 ± 0.1</td>
<td>1.04 ± 0.04</td>
<td>83/58</td>
<td>19/14</td>
</tr>
<tr>
<td></td>
<td>Ser49Gly</td>
<td>7.5 ± 0.1</td>
<td>0.99 ± 0.05</td>
<td>69/51</td>
<td>18/13</td>
</tr>
<tr>
<td></td>
<td>Gly49Gly</td>
<td>7.0 ± 0.1</td>
<td>1.08 ± 0.04</td>
<td>58/38</td>
<td>65/43</td>
</tr>
</tbody>
</table>

### Table 3. Inotropic Potencies and Intrinsic Activities of (−)-Norepinephrine at Polymorphic β1-Adrenergic Receptors in Right Atrial Trabeculae From Patients Not Having Long-Term Treatment With Beta-Blockers

<table>
<thead>
<tr>
<th>Effect</th>
<th>Genotype</th>
<th>−LogEC50</th>
<th>IA</th>
<th>%Ca2⁺</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Force</td>
<td>Ser49Ser</td>
<td>7.1 ± 0.1</td>
<td>1.00 ± 0.01</td>
<td>83.7 ± 3.1</td>
<td>21/14</td>
</tr>
<tr>
<td></td>
<td>Ser49Gly</td>
<td>7.0 ± 0.1</td>
<td>0.99 ± 0.02</td>
<td>87.8 ± 2.8</td>
<td>9/6</td>
</tr>
<tr>
<td>Force</td>
<td>Gly389Arg</td>
<td>7.1 ± 0.1</td>
<td>1.01 ± 0.02</td>
<td>84.9 ± 4.6</td>
<td>9/5</td>
</tr>
<tr>
<td></td>
<td>Arg389Arg</td>
<td>7.0 ± 0.1</td>
<td>0.99 ± 0.01</td>
<td>85.4 ± 2.9</td>
<td>21/15</td>
</tr>
</tbody>
</table>

Intrinsic activity (IA) was calculated as the fraction of the maximum effect of (−)-norepinephrine compared with the response of (−)-isoprotrenol (200 μM) causing maximal effects through β1-AR, and is expressed as a fraction of the maximum effect of (−)-norepinephrine with the response obtained by raising Ca2⁺ to 9.25 mM.

Abbreviations as in Tables 1 and 2.
be “normal” for the age group represented by CABG surgery patients. However, the conclusions reached in these studies for the effects of (−)-norepinephrine at Gly389Arg-beta_1-ARs differ from those obtained by Sandilands et al. (13). In the human right atrium from non-failing patients, they observed a 3.6-fold increase in potency of (−)-norepinephrine at Arg389- compared with Gly389-beta_1-ARs. However, before direct comparisons can be made with our study, experimental differences need to be considered, most notably the weaker basal contractile force of right atrial muscle (0.79 to 0.81 mN) than in our study (8.0 to 9.4 mN), which most probably resulted in the observed three- to eleven-fold lower (−)-norepinephrine potency and considerably smaller developed force (1.4 to 2.2 mN) than in our study (5.3 to 7.2 mN).

The observation of enhanced (−)-norepinephrine effect at human heart Arg389-beta_1-ARs observed by Sandilands et al. (13) is consistent with molecular studies at CHW-1102 fibroblast cells and COS-7 cells transfected with Gly389- or Arg389-beta_1-ARs. In these cell lines, basal and maximal isoproterenol-stimulated adenyl cyclase activities were greater for Arg389-beta_1-ARs, associated with “tighter,” more efficient coupling between receptor and Gs-alpha-protein (2). Collectively, these data also correctly predict differential phenotypes in patients with HF, where the presence of Gly389-beta_1-ARs is associated with reduced exercise capacity. The possibility of differential Gly389- and Arg389-beta_1-AR-mediated cardiostimulant effects could also be investigated directly in human ventricles from explanted hearts.

**Does long-term administration of beta-blockers nullify beta_1-AR polymorphic differences?** We observed a uniformity of (−)-norepinephrine cardiostimulant effects at Ser49- and Ser49Gly-beta_1-ARs and also at Arg389- and Arg389Gly-receptors in right atrial tissues of non-failing hearts from patients not receiving beta_1-blockers before surgery. This conclusion is also consistent with in vivo studies of exercise (endogenous (−)-norepinephrine) induced changes in HR, which were determined in young healthy volunteers not taking beta-blockers (8,9).

The antihypertensive effects (reduced systolic/diastolic blood pressure, reduced HR) of long-term administration of two beta_1-AR blockers (50 mg atenolol and 5 mg bisoprolol) were indistinguishable at Gly389Arg-beta_1-AR polymorphisms (14). Thus, taken together, it is unlikely that the long-term administration of the beta_1-blockers atenolol or metoprolol by patients in this study has caused differential effects at beta_1-AR polymorphisms.

Overall, we observed a small (2.4-fold) increase in potency for (−)-norepinephrine-mediated increases in contractile force in human right atrium from patients receiving atenolol or metoprolol compared with patients not receiving beta_1-blockers before surgery. In this respect there was no difference between atenolol or metoprolol (overall: \(-\log EC_{50}\) for (−)-norepinephrine-mediated increase in contractile force without consideration for polymorphic beta_1-ARs; atenolol: 7.42 ± 0.045, n = 95 tissues; metoprolol: 7.42 ± 0.05, n = 37 tissues, p = 1). This probably reflects an overall increase in stimulatory Gs-alpha-protein-coupled receptor function in human right atrium, previously observed for beta_2-ARs, H_2-receptors, 5-HT_1 receptors, and for RO363 at beta_1-ARs (11,15,16), which is uniform across all beta_1-AR polymorphisms.

**Study limitations.** Our investigation focused on two beta_1-AR polymorphisms, Ser49Gly and Gly389Arg, while there is evidence for five other polymorphisms (5). It could be argued that a complete phenotypic description of beta_1-AR function requires simultaneous consideration of all polymorphisms. Nevertheless, the uniformity and consistency of effects of (−)-norepinephrine across two polymorphisms in our study population were striking. This may not be the case as argued above for other pathologies where beta_1-AR function is attenuated. Our study did not find a mechanistic explanation for the increased survival benefit observed in congestive HF patients having Gly49-beta_1-ARs compared with patients homozygous for Ser49. Therefore, it may be necessary to investigate molecular mechanisms of Ser49Gly polymorphisms directly in failing human heart preparations (17), or in clonal cell lines (2).

**Conclusions.** Despite the presence of beta_1-AR polymorphisms in the human population, this study showed conservation of the effects of (−)-norepinephrine at two polymorphisms in the right atrium of adult non-failing hearts. Differences between polymorphic beta_1-ARs may be more apparent in pathologic conditions such as HF.

**Acknowledgments.** The authors thank Anne Carle, Donalee O’Brien, and surgical residents for obtaining informed consent from patients; anesthetists of the Department of Anesthetics for obtaining blood samples at the time of surgery; the cardiac surgeons of the Department of Cardiac Surgery for carefully dissecting right atrial appendages; Jill Larsen, Jessie Kelly, and Ainsley Tunnicliffe for extracting DNA; and Dr. Miranda Mortlock, PhD, for statistical advice.

**Reprint requests and correspondence:** Dr. Peter Molenaar, The National Heart Foundation and Prince Charles Hospital Foundation Cardiovascular Research Centre, Department of Medicine, University of Queensland, The Prince Charles Hospital, Chermside, Queensland, 4032, Australia. E-mail: p.molenaar@mailbox.uq.edu.au.

**REFERENCES**


