TRANSLATIONAL PHYSIOLOGY

Different expression of adrenceptors and GRKs in the human myocardium depends on heart failure ethiology and correlates to clinical variables

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1Departamento de Farmacología. Facultad de Farmacia. Universitat de València. Valencia, Spain; 2Servicio de Cardiología. Hospital La Fe. Valencia, Spain; and 3Instituto de Biomedicina de Valencia. Consejo Superior de Investigaciones Científicas. Valencia, Spain

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Montó F, Oliver E, Vicente D, Rueda J, Agüero J, Almenar L, Ivorra MD, Barettoni D, D’Ocon P. Different expression of adrenceptors and GRKs in the human myocardium depends on heart failure ethiology and correlates to clinical variables. Am J Physiol Heart Circ Physiol 303: H368–H376, 2012. First published June 8, 2012; doi:10.1152/ajpheart.01061.2011.—Downregulation of β1- adrenergic receptors (β1-ARs) and increased expression/function of G-protein-coupled receptor kinase 2 (GRK2) have been observed in human heart failure, but changes in expression of other ARs and GRKs have not been established. Another unresolved question is the incidence of these compensatory mechanisms depending on heart failure ethiology and treatment. To analyze these questions, we quantified the mRNA/protein expressions of six ARs (α1A, α1B, α1D, β1, β2, and β3) and three GRKs (GRK2, GRK3, and GRK5) in left (LV) and right ventricle (RV) from four donors, 10 patients with ischemic cardiomyopathy (IC), 14 patients with dilated cardiomyopathy (DC), and 10 patients with nonischemic, nondilated cardiopathies (NINDC). We correlated the changes in the expressions of ARs and GRKs with clinical variables such as left ventricular ejection fraction (LVEF) and left ventricular end-systolic and left ventricular end-diastolic diameter (LVESD and LVEDD, respectively). The main findings were 1) the expression of the α1A-AR in the LV positively correlates with LVEF; 2) the expression of GRK3 and GRK5 inversely correlates with LVESD and LVEDD, supporting previous observations about a protective role for both kinases in failing hearts; and 3) β1-AR expression is downregulated in the LV and RV of IC, in the LV of DC, and in the RV of NINDC. This difference, better than an increased expression of GRK2 (not observed in IC), determines the lower LVEF in IC and DC vs. NINDC.

G-protein-coupled receptor kinases

HUMAN HEART FAILURE (HF) is recognized as a major public health problem arising from multiple causes that will affect one in five adults, conferring elevated mortality rates (20). Regardless of the cause, multiple organ systems attempt to compensate for the deteriorating heart and the sympathetic nervous system responds to HF with increased activity, resulting in increased levels of catecholamines and in an enhanced stimulation of adrenergic receptors (ARs; 33). Consequently, catecholamines powerfully stimulate the heart function at the expense of overproportional increases in energy consumption (13). This is a nonrigid signaling system that adapts to continuous stimulation by reducing the abundance of β1-ARs and increasing the expression and enzymatic activity of G-protein-coupled receptor kinases (GRKs), which phosphorylate agonist-occupied receptors and facilitate their endocytosis and desensitization (26). As a result of these adaptations, cardiomyocytes in failing hearts (FH) lose their responsiveness to catecholamines over time (13).

In spite of this simple scenario, many fundamental questions remain unanswered. The heart expresses different β-ARs (β1, β2, and β3) and α1-ARs (α1A, α1B, and α1D) but not α2-ARs (7, 18, 19, 25, 40). Most experimental and clinical studies focus on the β1-ARs, but what happens with cardiac α1-ARs in HF? Furthermore, if elevated levels of catecholamines are the only reason for the previously described downregulation of β1-ARs, is the β1-AR subtype the only one that is downregulated in the FH?

The heart also expresses different GRKs (GRK2, GRK3, and GRK5). Differences among the GRKs in subcellular localization, activation mechanism, and receptor specificity suggest that they may play nonredundant modulatory roles in the heart (9, 10, 14, 15, 24). The rapid up- and downregulation of GRK2 and the less dynamic changes in the GRK5 expression are of particular interest, suggesting that GRK2 may function predominantly in the acute modulation of β-AR signaling, whereas GRK5 may prove more important for chronic modulation (20). Are these GRKs differentially regulated in HF?

Finally, does the regulation of ARs and GRKs differ in the left ventricle (LV) and right ventricle or depend on HF etiology?

To answer these questions, we quantified mRNA and protein expression of six ARs (α1A, α1B, α1D, β1, β2, and β3) and three GRKs (GRK2, GRK3, and GRK5) in nonfailing hearts (NHF) and in FH from patients with end-stage HF who had undergone a cardiac transplant. The group included patients with dilated cardiomyopathy or ischemic cardiomyopathy and patients with other forms of cardiomyopathy (nonischemic, nondilated). We analyzed the functional significance of the changes in the expression of ARs and GRKs by correlating them with the clinical variables related to the cardiac function such as left ventricular ejection fraction (LVEF), left ventricular end-systolic diameter (LVESD), and left ventricular end-diastolic diameter (LVEDD).
Table 1. Clinical data of patients with different types of heart failure

<table>
<thead>
<tr>
<th></th>
<th>IC</th>
<th>DC</th>
<th>NINDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>10/0</td>
<td>13/1</td>
<td>6/4</td>
</tr>
<tr>
<td>Age, yr</td>
<td>57 ± 2</td>
<td>50 ± 3</td>
<td>44 ± 7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.2 ± 1.3</td>
<td>26.9 ± 1.0</td>
<td>27.5 ± 2.2</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>16.8 ± 1.6</td>
<td>15.2 ± 2.0</td>
<td>21.6 ± 3.6*</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>63.2 ± 2.7</td>
<td>77.4 ± 3.2*</td>
<td>57.0 ± 3.2</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>54.8 ± 3.1</td>
<td>70.4 ± 2.6*</td>
<td>45.2 ± 4.0</td>
</tr>
<tr>
<td>Carvedilol, %</td>
<td>6 (60)</td>
<td>4 (29)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>Eniprenolactone, %</td>
<td>7 (70)</td>
<td>5 (50)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>ACEI, %</td>
<td>5 (50)</td>
<td>7 (50)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Diuretics, %</td>
<td>9 (90)</td>
<td>10 (71)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>Dobutamine, %</td>
<td>3 (30)</td>
<td>2 (14)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Dopamine, %</td>
<td>1 (10)</td>
<td>0</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Norepiphrenine, %</td>
<td>2 (20)</td>
<td>1 (7)</td>
<td>4 (40)</td>
</tr>
</tbody>
</table>

Values are means ± SE. DC, dilated cardiopathy; IC, ischemic cardiopathy; NINDC, nonischemic, nondilated cardiopathy; NFH, nonfailing heart (transplanted patients); M/F, male/female; BMI, body mass index; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; ACEI, angiotensin I-converting enzyme. *P < 0.01 vs. IC and DC; †P < 0.01 vs. IC and NINDC.

MATERIALS AND METHODS

This research conforms with the principles outlined in the Declaration of Helsinki and was performed with the approval of the Ethics Committee of the Universitat de Valencia and the Hospital Universitario La Fe de Valencia. Moreover, patients signed written consent before their inclusion in the study. Thirty-four heart transplant patients were consecutively recruited. All the patients received pharmacological therapy for HF with diuretics, carvedilol, angiotensin I-converting enzyme inhibitors, or intravenous β-agonist drugs (dobutamine), norepinephrine, or dopamine. As the dobutamine dose was modified according to patients’ hemodynamic status, a minimum duration of 7 days and ≥2.5 μg·kg⁻¹·min⁻¹ were required for the inclusion of patients. Patients with severe instability refractory to inotropic drugs were mechanically supported with an extracorporeal membrane oxygenation system as a bridge to urgent transplantation. As arterial hypertension and hypertensive heart disease are associated with increased GRK2 expression (9, 14, 24), we excluded those patients with previous long-standing hypertension or significant LV concentric hypertrophy according to echocardiographic records.

Samples of the LV and right ventricles from the myocardium were collected from explanted hearts. HF was due to ischemic heart disease (IC) in 10 patients, to dilated cardiomyopathy (DC) in 14 patients, and to other forms of cardiomyopathy (nonischemic, nondilated myocardopathies (NINDC)) in 10 patients. The functional parameters analyzed from the echocardiographic and hemodynamic studies were LVEF, LVEDD, and LVESD. Details of their drug regimens, together with the echocardiographic and hemodynamic data, are shown in Table 1. There were no significant differences in clinical variables among groups (IC, DC, or NINDC), except for the LVEF, which was significantly higher in the group including NINDC, and for the LVEDD and LVESD, respectively, which were significantly higher in the group of patients with DC (Table 1).

NFH tissues were obtained from donor hearts, which could not be transplanted for technical reasons and had no apparent signs of cardiac failure (n = 4).

Following heart extraction, a transmural sample for each patient was obtained from the posterior part of the LV adjacent to the interventricular septum and the right ventricular anterior wall in failing as well as NFH. The areas containing grossly visible scar tissue were avoided in patients with IC. Samples were immediately frozen in liquid nitrogen and stored at −80°C until processed.

Quantitative RT-PCR. The frozen samples were ground to powder in a mortar and divided into three portions. One portion was used to obtain total RNA as previously described (1, 2, 28), and the other two portions were reserved to obtain total proteins or binding assays. Total RNA was quantified and analyzed by running 1 μg of each sample by microfluidic electrophoresis using the Experion automated electrophoresis system (Bio-Rad, Madrid, Spain) following the manufacturer’s conditions. The software provided us with the RNA concentration (ng/ml), the 28S-to-18S ratio, and the RNA quality indicator (RQI, which measures RNA integrity by comparing the electropherogram of RNA samples to a series of standardized degraded RNA samples). The RQI method returns a number between 10 (intact RNA) and 1 (highly degraded RNA). In our samples, the mean value was 8.01 ± 0.11.

Total RNA using 500 ng and oligo(dT)16 as a primer (250 ng) in diethyl pyrocarbonate-treated water were preheated to 70°C and cooled on ice for cDNA synthesis. The reactions (20 μl) contained ImProm-II TM reaction buffer, 3 mM MgCl₂, 20 U of recombinant RNasin ribonuclease inhibitor (Promega, Madison, WI), 0.5 mM of each deoxynucleoside triphosphate, and 1 μl of ImProm-II TM reverse transcriptase (Promega) and were incubated at 25°C for 5 min (annealing step), followed by an extension step at 42°C for 60 min and a final step at 70°C for 15 min (heat-inactivate). The mRNAs encoding the three α1 adrenerceptor subtypes (α1A, α1B, and α1D), the β-adrenerceptors (β1, β2, and β3), the three GRKs mainly expressed in the myocardium (GRK2, GRK3, and GRK5) and glyceraldehyde-3-phosphate dehydrogenase (Gapdh) and TATA-box binding protein (Tbp) as internal standards, were quantified by TaqMan real-time RT-PCR with a GeneAmp 7500 Fast System (Applied Biosystems, Carlsbad, CA). We analyzed (in duplicate reactions) a 10-fold dilution of the RT reaction of each sample using the TaqMan Gene Expression Assays (Applied Biosystems).

The specific primer-probes were α1A-AR (Hs00169124_m1), α1B-AR (Hs00171263_m1), α1D-AR (Hs00169865_m1), β1-AR (Hs00265096_s1), β2-AR (Hs00240532_s1), β3 (Hs00609046_m1), GRK2 (Hs00176395_m1) GRK3 (Hs00178266_m1) GRK5 (Hs00173839_m1), Gapdh (Hs99999905_m1), and Tbp (Hs99999910_m1) (Applied Biosystems). Real-time PCR reactions were done in 25 μl with TaqMan universal PCR master mix (Applied Biosystems), including 5 μl of diluted RT reaction, and 1.25 μl of 20× TaqMan gene expression assay mix (250 nmol/l for the probe and 900 nmol/l for each primer). cDNA was amplified following the manufacturer’s conditions: one initial hold-step at 95°C for 10 min, a second step with 40 cycles, 15 s at 95°C (denaturation), and 1 min at 60°C (annealing/extension). The targets and reference (Gapdh and Tbp) were amplified in parallel reactions.

As several studies (6) have concluded that it is difficult to identify general reference genes, which can be used in all experimental settings, and that validation of the chosen reference gene(s) is important for each experimental setting, we used two candidate genes (Gapdh and Tbp) to test their utility as an internal reference in our

Fig. 1. Graphical representation of the linear regression observed between the expression of the two reference genes glyceraldehyde-3-phosphate dehydrogenase (Gapdh) and TATA-box binding protein (Tbp) in human left ventricle. Data represent the cycle number of Gapdh and Tbp in each patient.
patients. Figure 1 shows the close correlation found in the expression level of each reference gene in the LV. Thus under our experimental conditions, Gapdh is a suitable reference gene, and we chose it as housekeeping gene to compare our data to previous observations in the failing and the nonfailing myocardia. Then, the cycle threshold (Ct) values obtained for each gene were referenced to Gapdh and converted into the linear form using the term $2^{-\Delta Ct}$ as a value directly proportional to the mRNA copy number (21).

**Western blot.** To obtain total proteins, a portion of each previously pulverized frozen sample was homogenized with a Microson XL 2000 ultrasonic liquid processor in ice-cooled RIPA lysis buffer (50 mmol/l HEPES pH 7.5, 150 mmol/l NaCl, 10% glicerol, 1.5 mmol/l MgCl2, 0.1% SDS, 1 mmol/l EGTA, 100 mmol/l NaF, 1% Triton X100, and 1% sodium deoxycholate) containing protease inhibitor cocktail (Complete, Roche Diagnostics, Mannheim, Germany). This was centrifuged at 16,100 $g$ for 15 min at 4°C. The protein concentration was determined by the Bradford method (Bio-Rad Laboratories, Hercules, CA). Protein extracts (150 μg) were loaded onto 10% SDS-polyacrylamide gels, and electrophoresed proteins were transferred to PVDF membranes 2 h at 375 mA, using a liquid Mini Trans-Blot electrophoretic transfer cell system (Bio-Rad Laboratories). Membranes were blocked in 5% nonfat dried milk in PBS containing 0.1% Tween 20 for 1 h at room temperature with gentle agitation. Membranes were then washed and incubated with rabbit polyclonal antibodies against GRK2 (sc-562, 1:250), GRK3 (sc-563, 1:100), and GRK5 (sc-565, 1:100) from Santa Cruz Biotechnology and with rabbit anti-GAPDH (G9545, 1:5000) from Sigma-Aldrich, as a loading control diluted in blocking solution at 4°C overnight. Membranes were then washed three times, incubated with rabbit anti-goat IgG horseradish peroxidase-conjugated secondary antibody (Jackson Immuno-Research Laboratories, West Grove, PA) at 1:2500 or with donkey anti-rabbit IgG horseradish peroxidase-conjugated (Amersham Biosciences, Buckinghamshire, UK) at 1:2500 or 1:3000 for 50 min at room temperature, and washed extensively before incubation with ECL Western blotting detection reagent (Amersham Biosciences). Membranes were immediately documented and quantified with an Autochemi BioImaging System using the Labworks 4.6 capture software (Ultra-Violet Products, Cambridge, UK).

**Radioligand binding assays.** A portion of each previously pulverized frozen sample (2,000 μg protein) was incubated in duplicate for 60 min at 37°C with nine concentrations of [3H]CGP 12177 (0.1–20 nM) in 50 mM Tris·HCl (pH 7.5). Experiments were terminated by aging frozen sample (2,000 $g$) at 37°C with nine concentrations of [3H]CGP 12177 (0.1–20 nM) in 50 mM Tris·HCl (pH 7.5). Experiments were terminated by rapid filtration through fiberglass filters (GF 52; Schleicher and Schuell) presoaked in 0.3% polyethyleneimine using a Brandel cell harvesting System using the Labworks 4.6 capture software (Ultra-Violet Products, Cambridge, UK).

**Statistical analysis.** The statistical analysis was performed using the Graph Pad Software. Data were expressed as means ± S.E. One-way ANOVA and Student’s t-test were performed. Linear regression and Pearson’s correlation test were used to establish associations between variables. Significance was defined as $P < 0.05$.

**RESULTS**

**Expression level of ARs and GRKs in human myocardium.** In all samples, the most expressed ARs were β1 and β2, followed by the α1A-subtype. The other three ARs, α1B, α1D, and β3, were a minority. A significant decrease in the expression of β1-AR was observed in both chambers from FH, compared with NFH (Table 2). The expression of the other ARs did not change in FH but, due to a drastic drop in the β1-AR expression, their relative proportion significantly increased (from 25.98 ± 8.685 of total mRNA for the β2-ARs determined in the LV of NFH to 48.8 ± 2.1% in FH; from 5.37 ± 0.32% for the α1A-AR in NFH to 10.5 ± 0.6% in FH). These results suggest that the relative contribution of the β2- and α1A- subtypes to the adrenergic response could be increased in FH. Among the GRKs, GRK5 was the most abundant, followed by GRK2 and GRK3. A slight, but not statistically significant, increase in the expression of the three GRKs was found between FH and NFH (Table 2). While GRK3 and GRK5 were uniformly distributed in both chambers, GRK2 appeared to be more expressed in the LV than in the right ventricle of FH (Table 2).

A linear regression analysis was performed for a given AR-subtype between the mRNA values obtained in the LV and the right ventricles of the same patient. As Fig. 2 shows, a significant relationship was found between chambers, except for β1-AR, which suggests a selective regulation of the expression of this receptor in each chamber.

**Correlation between the expression levels of ARs and GRKs in FH and clinical variables.** A positive correlation between the LVEF and the expression level of β1 and β2-ARs determined in the LV from FH was observed (Fig. 3, A and B). Furthermore as Fig. 3C depicts, a similar correlation was found between LVEF and the mRNA level of α1A-AR in the LV. There was also a significant direct relationship between the GRK5 expression in the LV and LVEF (Fig. 3D). In addition, the GRK3 and GRK5 expression levels in the LV significantly and inversely correlated with LVESD and LVEDD (Fig. 4).

**Comparative analysis of the expression level of ARs and GRKs depending on the pharmacological treatment.** Treatment with the α1/β-AR antagonist carvedilol or the agonists dobutamine or norepinephrine could affect the expression of adrenoceptors and GRKs in the human heart; a comparative analysis was then performed in subgroups of patients receiving or not the above-mentioned drugs. Treatment with adrenoceptor agonists did not significantly modify the expression of ARs or GRKs in our group of patients (results not shown). Only the expression of α1A-ARs changed in patients under treatment with carvedilol. As Fig. 5 shows, a significant increase in the expression of α1A-ARs was observed in the right ventricle, suggesting an upregulation of this subtype due to continuous

**Table 2. Expression of ARs and GRKs in the human left and right ventricle from failing and nonfailing hearts**

<table>
<thead>
<tr>
<th></th>
<th>Nonfailing Heart</th>
<th>Failing Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left ventricle</td>
<td>Left ventricle</td>
</tr>
<tr>
<td>α1A</td>
<td>12.14 ± 3.01</td>
<td>14.77 ± 1.52</td>
</tr>
<tr>
<td>α1B</td>
<td>0.56 ± 0.14</td>
<td>0.707 ± 0.085</td>
</tr>
<tr>
<td>α1D</td>
<td>0.20 ± 0.08</td>
<td>0.427 ± 0.058</td>
</tr>
<tr>
<td>β1</td>
<td>181.37 ± 71.4</td>
<td>65.75 ± 8.71*</td>
</tr>
<tr>
<td>β2</td>
<td>44.49 ± 4.61</td>
<td>68.20 ± 5.79</td>
</tr>
<tr>
<td>β3</td>
<td>0.11 ± 0.04</td>
<td>0.154 ± 0.029</td>
</tr>
<tr>
<td>GRK2</td>
<td>23.80 ± 1.72</td>
<td>31.00 ± 2.79</td>
</tr>
<tr>
<td>GRK3</td>
<td>5.90 ± 0.49</td>
<td>10.55 ± 1.15</td>
</tr>
<tr>
<td>GRK5</td>
<td>56.38 ± 3.62</td>
<td>78.24 ± 6.15</td>
</tr>
</tbody>
</table>

Values are means ± SE. mRNA levels of each gene expressed as $2^{-\Delta Ct}$ with GAPDH as the housekeeping gene. Independent samples t-test and ANOVA were performed. AR, adrenergic receptor; GRK, G-protein-coupled receptor. *P < 0.01 vs. left ventricle from nonfailing heart.
blockade by the antagonist. No significant changes were observed in \( \beta_1 \)- or \( \beta_2 \)-ARs (Fig. 5) nor in GRK expression with carvedilol treatment (results not shown).

**Comparative analysis of the expression level of ARs and GRKs in different HF types.** We performed a comparative analysis to determine if the expression levels of ARs and GRKs change depending on the type of HF: IC, DC, or NINDC cardiomyopathy. As Fig. 6A illustrates, the expression of \( \alpha_{1A} \)-ARs was equally distributed in both the LV and right ventricles of the three subgroups and no significant differences were observed in FH vs. NFH. Similar results were obtained for the minority \( \alpha_{1B} \) and \( \alpha_{1D} \)-ARs (data not shown).

A lower mRNA of \( \beta_1 \)-AR was observed in the LV of IC and DC vs. NFH, but no significant decrease in \( \beta_1 \)-AR expression was found in NINDC. The right ventricle exhibited a different pattern of changes, where the expression level of \( \beta_1 \)-AR in the NINDC was similar to the IC group but lower than NFH. In this case, no significant changes in the expression of \( \beta_1 \)-AR were observed in the right ventricle of DC patients (Fig. 6B). The distribution of \( \beta_2 \) exhibited great variability among the three patient subgroups (Fig. 6C), and the same occurred with \( \beta_3 \)-AR (results not shown), but the mean values were not statistically different from NFH.

Binding of the \( \beta \)-AR selective radioligand \([\text{H}]\text{CGP12177}\) provides evidence for a decrease in the protein expression of \( \beta \)-ARs (Fig. 6D).

Major changes in the expression levels of GRKs were observed depending on the subgroup of patients and the pathology. In IC patients, gene expression of the three GRKs isoforms did not statistically differ from NFH (Fig. 7, A, C, and E). However, in the LV of DC and NINDC, a significant increase in the mRNA expression of GRK2 was noted vs. NFH (Fig. 7A). GRK3 also increased, but the difference did not reach statistical significance (Fig. 7B). Finally, GRK5 significantly increased in the LV and right ventricles of NINDC (Fig. 7C). The protein expression determined by immunoblotting...
reproduces the observed changes in mRNA levels (Fig. 7, B, D, and F).

DISCUSSION

Our study describes the expressions of the α1- and β-AR subtypes and the three GRKs (GRK2, GRK3, and GRK5) involved in their regulation in different types of HF and compares them to the expressions of the same genes in NFH to answer the questions that follow.

What happens with cardiac α1-ARs in HF? The density of human cardiac α1-AR is 10–15% of that of β-AR (7, 19, 40, 41). Although the present data show no changes in the expression of α1-ARs in human HF, a relative increase in its proportion at the expense of a lower β-AR density is observed. Among the three subtypes, α1B predominates in rodents but, and according to Jensen et al. (19), our results demonstrate that the mRNA level for the α1A-subtype in the human myocardium is, by far, the most abundant. In addition, the expression of α1A-AR in the LV but not that of α1B or α1D, significantly correlates with the LVEF, suggesting that α1A-ARs may contribute to maintain cardiac inotropy in the FH. Although α1-ARs are not generally considered to be major regulators of the cardiac contractile function under physiological conditions, they are thought to have more influence under pathological conditions, such as HF (18). The correlation found between the LVEF and mRNA levels for α1A-AR in the human ventricle, not previously described, suggests that some degree of α1A activation could be beneficial in this pathology, as previously proposed in mouse (27) and humans (32, 39). In addition, previous data have associated α1A-ARs with cardioprotection, whereas α1B-ARs are associated with cardiac growth and hypertrophy (16, 18, 41). These beneficial effects of α1A-AR activation in failing human hearts could explain the harmful results of α1-blockade in clinical trials such as the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack (3).

Is the β1-AR subtype the only one to be downregulated in the FH? In human NFH, β1-AR constitutes 70–80% of the total β-ARs, while β2-AR constitutes the remaining 20–30% with β3-AR being poorly expressed (7, 25, 42) In FH, the β1-to-β2 ratio decreases to 50/50 or drops even lower (23, 29, 36), and this change is attributed to selective β1-AR downregulation (5, 7, 12, 22). Similar results are found when analyzing the mRNA levels in end-stage FH (the β1-to-β2 ratio at ~40/60), thus confirming the existence of a specific mechanism that dramatically downregulates the β1-AR expression in HF.

What could be the reason for this specific downregulation of β1-ARs in the FH? In patients with end-stage HF who exhibit increased levels of catecolamines, an overactivity of cardiac β1-ARs has deleterious consequences for the heart as has been shown in transgenic mice (42, 43, 44). Accordingly, the specific decrease in the expression and functionality of β1-AR could be a protective mechanism for the damaged heart (13). Moreover, the close correlation found in the expression of the other five adrenoceptors between the LV and right ventricles, not observed for β1-AR, corroborates with the existence of a specific regulation for this subtype in each chamber. In this scenario, β2-AR could play a predominant functional role and our results show that in FH, LVEF directly correlates not only with β1 but also with the expression of β2- and α1A-AR, indicating that the three subtypes could play a role in regulating cardiac functionality in the failing myocardium.

Are the three GRKs present in the human heart (GRK2, GRK3, and GRK5) differentially regulated in HF? Abundance of the three different GRKs characterized in the heart follows the order GRK5 > GRK2 ≥ GRK3 and corroborates previous data in the human heart (20, 33, 35). A nonsignificant increase in the expression of the three GRKs was observed in HF, but an interesting result not previously evidenced is the higher expression of GRK3 and GRK5 associated with a lower degree of the LVESD or LVEDD, adding new information to previous findings supporting a protective role for GRK3 in the cardiovascular system (28, 38) and contributing to a better understanding of the position of the GRK3 gene in a locus on a chromosome associated with LV mass and contractility (4). The inverse correlation found between the GRK5 expression (a kinase that modulates cardiac β1 activity) and cardiac hypertrophy supports clinical evidence for a protective role of a GRK5 polymorphism in HF that inhibits β1-AR signaling more effectively (20).

As the present results evidence, the higher expression of both GRK3 and GRK5 correlates with a better clinical status focusing on LVESD, LVEDD or LVEF. A possible expla-
nation stems from the following observation: the Gs/cAMP pathway, which mediates inotropic and chronotropic responses, is stimulated by β2-ARs in the heart, but with lower efficiency than β1-ARs (8). Unlike β1-AR, β2-AR also interacts with an inhibitory Gt protein, with the phosphorylated form of β2-AR apparently doing so preferentially (29). The β2-AR-Gt signaling pathway plays a crucial role in cardioprotection against apoptotic death of cardiomyocytes (44, 45). Thus increased GRK activity could result in an increased phosphorylation of the β2-subtype then favoring the cardioprotective mechanism mediated by the β2/Gt pathway.

Does the regulation of ARs and GRKs differ depending on HF treatment and/or etiology? No significant differences were found in the expression of ARs and GRKs depending on the drug regimen of patients. Only the subgroup treated with the α1/β-AR antagonist carvedilol exhibits an upregulation of the α1A subtype in the right ventricle, which can be interpreted as consequence of a continuous blockade of this subtype and could be involved in the protective effect of carvedilol on HF patients. No significant differences were found in the expression of β-ARs indicating that carvedilol treatment was not a determinant factor in the changes observed in FH.

Among the three subgroups of cardiopathies analyzed (patients with DC), patients with IC, and patients with NINDC, the cardiac explants from patients with NINDC exhibit the greatest β1-AR expression in the LV, which is slightly lower but not significantly different to NFH, and could be the reason for the significantly higher LVEF found in this patient subgroup. Interestingly in NINDC, the downregulation of β1-AR is greater in the right cavity. As the NINDC group presents a better ejection fraction than IC or DC, together with a poor therapeutic response to β-blockers, future work to specifically address HF patients with a preserved ejection fraction could determine the clinical implication of the more marked decrease in the expression of the β1-ARs in the right ventricle of this patient subgroup and its consequences for the pharmacological management of HF with a preserved ejection fraction.

Conversely, patients with DC exhibit a downregulation of the β1-AR only in the LV. Finally, the cardiac explants from patients with DC present a lower expression of β1-ARs in both LV and right ventricles. Then, a selective downregulation of the β1-AR is observed depending on HF etiology. The protective role of the previously discussed β1-AR downregulation is further supported by the fact that among the three types of HF analyzed, the IC patients who have had considerable, acute cardiac injury exhibit the lowest β1-ARs expression in both LV and right ventricles.

In DC and NINDC, an increased expression of the GRK2 is observed, thus corroborating previous studies describing the upregulation of GRK2 in HF (9, 11, 14, 17, 34, 35, 37). The rapid up- and downregulation of GRK2 observed in previous studies (20) suggests that GRK2 may function predominantly in the acute modulation of β-AR signaling. In this case, the GRK2 upregulation observed in DC and NINDC could relate to the chronic elevation of the sympathetic nervous system activity in HF (30), to the clinical severity of HF, or to pharmacological treatment (2). Therefore, a more profound analysis of GRK2 expression in relation to circulating catecholamines or treatment with β-blockers or dobutamine, dopamine, and norepinephrine could be the aim of a future work.

Nevertheless, an interesting result is noted in the IC patients group where the expression of the three GRKs is not...
statistically different to NFH but is lower than DC and NINDC. Therefore, those IC patients with a lower expression of GRK2 and β1-ARs exhibit markedly altered cardiac activity, as the LVEF indicates. Thus the downregulation of β1-ARs, and not the upregulation of GRK2, appears to be the crucial maladaptive mechanism that determines the characteristic lower ejection fraction in IC patients. The results obtained for NINDC corroborate this hypothesis since this group exhibits a higher expression of β1-ARs in the LV together with a higher LVEF, irrespectively of the increased expressions of GRK2 and GRK5 in this chamber.

Taken together, the novel observations of the present work are 1) α1A-AR is the main expressed α1-subtype in human FH, and its expression in the LV positively correlates with LVEF; 2) the expression of GRK3 and GRK5 in the LV inversely correlates with LVESD and LVEDD, supporting previous observations about a protective role for both kinases in FH; and 3) β1-ARs expression is downregulated in the LV and right ventricle of IC, in the LV of DC, and in the right ventricle of NINDC. This difference, more than an increased expression of GRK2, not found in IC patients, determines the lower LVEF observed in IC and DC vs. NINDC.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
REFERENCES


