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Sex-related differences in age-associated downregulation of human ventricular myocardial β_1-adrenergic receptors

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BACKGROUND: With increasing age, human ventricular myocardium exhibits selective downregulation of β_1-adrenergic receptors (β_1-ARs). We tested the hypothesis that sex differences exist in age-related changes in β_1-ARs.

METHODS: Left (LV) and right (RV) ventricular tissue was obtained from 61 unplaceable potential organ donor hearts ages 1 to 71 years with no known cardiac history and from LVs removed from 56 transplant recipients with idiopathic dilated cardiomyopathy. β_1-AR and β_2-AR densities, the frequency of β_1-*AR389* gene variants, and β-AR function were determined.

RESULTS: Sex had a marked effect on the age-related decrease in β_1-ARs. Female LVs had more pronounced downregulation (by 42% [$p < 0.001$] vs 22% [$p = 0.21$] in 31 male LVs) comparing the youngest (average age, 15.3 ± 5.5 years) to the oldest (average age, 50.8 ± 9.1 years) sub-groups. On regression analyses, female LVs exhibited a closer relationship between β_1-AR density and age ($r = –0.78$, $p < 0.001$ vs $r = –0.46$, $p = 0.009$ in males), with a second-degree polynomial yielding the best fit. There was no statistically significant relationship of β_1-ARs to age in female or male idiopathic dilated cardiomyopathy LVs.

CONCLUSIONS: Sex affects age-related β-AR downregulation in normal human ventricles, with females exhibiting more profound decreases with increasing age. The curvilinear relationship between age and receptor density that plateaus around age 40 in women suggests an effect of sex hormones on β_1-AR expression in the human heart.

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Myocardial contractile state is regulated beat-to-beat by cardiac adrenergic activity[1] via signal transduction through ventricular cardiac myocyte β-adrenergic receptors (β-ARs).[2] In human ventricles, β_1-ARs are dominant,[2] but β_2-ARs are present in lower density and may also mediate

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changes in heart rate and contractility. β_1-ARs are also proximally positioned in an extensive gene regulatory network that includes Ca^{2+} handling, contractile protein, metabolism, and neurohormonal signaling categories.[3,4] The chronic activation of this β_1-AR gene network is a major regulator of contractile function and myocardial growth, generally producing decreased function and hypertrophy.[3] Inhibition of this gene network likely explains much of the clinical benefit of β-blocking agents in chronic heart failure with reduced ejection fraction (HFrEF).[3,5,6]

Major influences on β_1-AR expression in human ventricular myocardium include age,[7,8] chronic administration of β-agonists[9] or antagonists,[10] thyroid hormone,[11,12] and HFrEF,[2,13] the latter likely through locally regulated chronic increases in adrenergic activity.[14] Modulators of β_1-AR function independent of protein expression include the *ADRB1* Arg389Gly genotype[15,16] and acute exposure to adrenergic stimulation.[17,18] Another possible regulator of human myocardial β_1-AR expression is sex, because there are sex and age interactions for plasma norepinephrine levels,[19–21] skeletal,[22] or cardiac[23] muscle sympathetic nerve activities, and exercise heart rate.[24] In addition, sex and age clearly interact in myocardial hypertrophy induction[25–27] and development of heart failure,[28,29] both part of the biologic response to the β_1-AR signaling gene network. Finally, effects of age and sex on cardiac β_1-AR expression in animal models have yielded mixed results.[30–33] In the current investigation, we hypothesized that sex and age interact to influence β_1-AR protein expression in human ventricular myocardium.

Methods

Tissue procurement

Explanted human hearts were obtained from organ procurement agencies and cardiac transplant programs between the years 1981 and 2001. During this time, the organ donor pool was relatively plentiful, and most transplant candidates were not being treated with β-blocking agents, intravenous inotropic agents, or mechanical assist devices, treatments that might have direct effects on β-AR expression or function. Explanted idiopathic dilated cardiomyopathy (IDC) hearts with end stage HFrEF were selected for the absence of known β-blocker, β-agonist, or type 3 phosphodiesterase inhibitor exposure before transplant. With the exception of genotyping, all assays reported were performed in within 6 months of cardiac explant. Right ventricular (RV) trabeculae force-development studies were performed at the time of explantation.[13,14]

Organ donors

Human hearts with no cardiac history (non-heart failure [NHF] hearts) were obtained via the Northern California, Intermountain, and Colorado organ procurement agencies in collaboration with the Stanford, Utah, and Colorado cardiac transplant programs. Sixty-one hearts from potential donors were obtained from 30 female and 31 male organ donors, aged 1 to 71 years. Potential donors had been excluded from organ donation because no recipient could be identified with compatible age, body size, or AB blood type after at least 2 placement attempts using the United Network for Organ Sharing. Written consent for organ donation for research purposes was obtained from a family member.

Thirty hearts were procured within a 50-mile radius of the processing research laboratory, had an ischemic time of $\leq$45 minutes, and were designated on-site (OS) explants. OS hearts were rapidly explanted and immediately immersed in ice-cold, oxygenated physiologic salt solution.[13,34] Sixteen hearts were designated as remote-site (RS) explants and were transported for periods $>$45 minutes $<$2.5 hours, packed in ice after cardioplegia, and immersed in ice-cold, oxygenated physiologic salt solution at explantation.[13]

In 46 hearts, an echocardiogram obtained as a routine part of the organ donation process[35] or visual and manual inspection by the donor heart surgical team revealed normal left ventricular (LV) function, defined on echocardiogram as a shortening fraction $\geq$ 25%. Fifteen hearts had donor heart dysfunction (DHD), with an LV fractional shortening of $<$ 25%. Fourteen of the 15 DHD hearts were locally procured. In only 2 donors had β-adrenergic agonists other than dopamine been administered, in both cases 4 μg/kg/min dobutamine.

End-stage failing hearts with IDC

Fifty-six hearts were removed from 14 female and 42 males with IDC and end-stage HFrEF, ages 10 to 61 years, at the time of transplantation. These transplant recipients had no obstructive coronary disease $>$ 50% in large coronary arteries and no history of myocardial infarction, valvular heart disease, or inflammatory heart disease. Mean LV ejection fraction (LVEF) was 15.2% $\pm$ 5.3% (standard deviation). IDC patients were treated variably with angiotensin-converting enzyme inhibitors or receptor-blocking agents, diuretics, and digoxin at the time of cardiac transplantation. One patient was on a blinded study medication in a randomized placebo controlled β-blocker study. No patients were being treated with an open label β-blocker, type 3 phosphodiesterase inhibitors, or β-agonists. IDC hearts were handled and processed identically to OS organ donor hearts.

β-AR measurements

All organ donor and IDC LVs had β-AR measurements, and 55 donors also had RV measurements. Ventricular free wall samples were homogenized, and membranes were prepared and stored at –80°C.[13,14] The total population of β-AR and the percentages of β_1- and β_2-receptors were reported as previously described.[13]

Adenylyl cyclase activity

In a sub-set of hearts (38 organ donor, 37 IDC), adenylyl cyclase (AC) activity and maximum response to isoproterenol were measured as previously described[36] of 10 mmol/L NaF.

Force development in RV trabeculae

In a sub-set of hearts (41 organ donor, 32 IDC), the response of 1-4 isolated human RV trabeculae was assessed, as previously described.[13]