ALTERATIONS IN HUMAN CARDIAC β -ADRENOCEPTOR DENSITY WITH AGING

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Abstract : Purpose : We designed this study to investigate the mechanism of reduced cardiac β -adrenoceptor function that is associated with aging.

Materials and Methods : We assessed the density and affinity of β -adrenoceptors by (-)-[¹²⁵I]iodocyanopindolol binding in the right atrial appendages obtained from 40 patients of varying age (range : 2 to 56 years) undergoing open-heart surgery. The mean right atrial pressure and cardiothoracic ratio were measured in all of the patients.

Results : There was a significant negative correlation between human right atrial β -adrenoceptor density and patient age ($r=0.575$, $p<0.001$). Neither preoperative mean right atrial pressure nor preoperative cardiothoracic ratio was correlated with β -adrenoceptor density. The affinity of β -adrenoceptors did not correlate with patient age.

Conclusions : The decrease in human cardiac β -adrenoceptor function associated with increasing age is due, in part, to the reduced cardiac β -adrenoceptor density.

Key words : Human heart, β -adrenoceptors, Aging
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INTRODUCTION

Cardiac β -adrenoceptor (β AR) function has been shown to decrease with increasing age^{1~6}). The mechanism underlying the reduced β AR function is believed to be due not to reduced β AR density, but to diminished receptor- and postreceptor-mediated activation of adenylyl cyclase^{7~10}). However, reduced β AR density and associated diminished postreceptor-mediated adenylyl cyclase activation has also been reported in the aged rat myocardium¹¹). Lymphocytes have been used to study changes in the β AR system in aging humans^{12~20}). However, it has been shown that β AR changes in the human heart are not reflected by changes in circulating lymphocyte β AR²¹). There is currently little data regarding age-related changes in the human cardiac β AR system²²). Therefore, in order

to evaluate age-related alterations in the human cardiac β AR system, we determined the β AR density in the right atrium from patients of different ages.

MATERIALS AND METHODS

Study patients

Forty patients (17 females and 23 males; mean age : 25 ± 3 years, range : 2 to 56 years) undergoing open-heart surgery were enrolled in this study. Twenty-six of the patients had atrial septal defects, and 14 patients had ventricular septal defects. All of the patients were in NYHA functional class I or II. None of the patients had been treated with either beta adrenoceptor agonists or antagonists for at least 3 weeks before surgery. All of the patients gave informed written consent to participate in the study.

Induction and maintenance of anesthesia were

accomplished with high-dose morphine hydrochloride. Mechanical ventilation was maintained with 100% O₂. After systemic heparinization, nonpulsatile cardiopulmonary bypass (CPB) was initiated at a flow of 2.4L/min/m².

In patients with atrial septal defects, repair was performed under electrically-induced ventricular fibrillation without aortic cross-clamping. In patients with ventricular septal defects, intracardiac repair was performed after the aorta was cross-clamped and the heart arrested with cold (4°C) St. Thomas' cardioplegic solution (10ml/kg) infused into the aortic root. Moderate continuous hypothermic perfusion was maintained at a mean temperature of 28°C. A portion of the right atrial appendage was excised immediately before the initiation of CPB. The right atrial specimens were quickly frozen and stored at -80°C.

Mean right atrial pressure (MRAP) was measured during the preoperative diagnostic cardiac catheterization. The cardiothoracic ratio (CTR) was measured on the preoperative chest roentgenogram.

Crude membrane preparation

Human right atrial specimens were minced with scissors and homogenized in ice-cold buffer (20mM Tris-HCl, 0.9% NaCl; pH7.5 at 4°C) with a Polytron homogenizer (Brinkman Instruments, Westbury, New York) at setting 8 for 20 seconds. The homogenate was filtered through two layers of medical gauze and homogenized with a Polytron. After centrifugation at 20000g for 10 minutes at 4°C, the pellet was resuspended in ice-cold Tris-HCl buffer, using a Potter-Elvehjem homogenizer, at a final protein concentration of 250 to 450µg/ml. The protein content was determined using the method of Lowry et al.²³⁾ using bovine serum albumin as the standard. The membranes were then used immediately for the radioligand binding assay.

Radioligand binding assay

The βAR radioligand binding assay was performed with (-)-[¹²⁵I] iodocyanopindolol (ICYP)²⁴⁾. The crude membranes (150µl) were incubated in duplicate for 40 minutes at 37°C with various concentrations of ICYP (25 to 400pM) in 50µl of Tris-HCl buffer, final volume of 250µl, to obtain total binding. Nonspecific binding was determined in duplicate in the presence of 10µM l-propranolol. After incubation in a shaking water bath, the binding reaction was terminated by rapid vacuum filtration through glass fiber filters (GF/C). The filters were subsequently washed three times with 5 ml of ice-cold 20mM Tris-HCl buffer. Filters were then counted in a gamma counter.

Specific binding was defined as the difference

between total and nonspecific binding (Fig.1). Receptor number (B_{max}) and affinity (K_D) were determined by Scatchard analysis⁽²⁵⁾ using linear regression performed by least-squares analysis (Fig.1). Receptor density is expressed in femtomoles of receptor per milligram of membrane protein. To evaluate the stereospecificity of the [¹²⁵I] ICYP binding site, competition studies were performed by incubating the crude membrane with 200pM [¹²⁵I] ICYP and increasing amounts (10⁻⁸ to 10⁻³M) of unlabeled (+) or (-) propranolol. To evaluate the specificity of the [¹²⁵I] ICYP binding site, competition studies were also performed by incubating the crude membrane with 200pM [¹²⁵I] ICYP and increasing amounts (10⁻¹⁰ to 10⁻²M) of unlabeled agonists ((-) isoproterenol, (-) epinephrine, or (-) norepinephrine), or antagonists (atenolol, metoprolol, pindolol, or timolol).

Fig. 1. Representative saturation-binding isotherm of (-)-[¹²⁵I]iodocyanopindolol (ICYP) binding to the crude membrane from human right atrial myocardium. ●=total binding; ○=binding in the presence of 10µM l-propranolol (nonspecific binding); * =derived specific binding. The inset is a Scatchard plot of specific binding data (K_D=20.5pM; B_{max}=42.0 fmol/mg protein).

Statistical analysis

Correlation coefficients were calculated and tested for significance using StatView statistical software (version 4.5; Abacus Concepts, Inc., Berkeley, CA). A value of p<0.05 was considered statistically significant.

RESULTS

Inhibition of [¹²⁵I] ICYP binding by (-) propranolol (IC₅₀ of 5.0×10⁻⁷M) was 30 times more potent than that of (+) propranolol (IC₅₀ of 1.4×10⁻⁵M), indicating that the binding of [¹²⁵I] ICYP to membranes is stereospecific (Fig.2). Competition with

Fig. 2. The competition of unlabeled (+)- or (-)-propranolol for $[^{125}\text{I}]$ ICYP binding sites at a ICYP concentration of 200pM. Left ordinate: Percent inhibition of $[^{125}\text{I}]$ ICYP bound. Abscissa: $-\log$ (+)- or (-)- propranolol concentration in M. $\bullet$ =(+)-propranolol; $\circ$ =(−)-propranolol. $[^{125}\text{I}]$ ICYP: (−)- $[^{125}\text{I}]$ iodocyanopindolol

Fig. 3. The competition of unlabeled agonists for $[^{125}\text{I}]$ ICYP binding sites at a ICYP concentration of 200pM. Left ordinate: Percent inhibition of $[^{125}\text{I}]$ ICYP bound. Abscissa: $-\log$ agonist concentration in M. $\bullet$ =(−)-norepinephrine; $\circ$ =(−)-epinephrine; $*$ =(−)-isoproterenol. $[^{125}\text{I}]$ ICYP: (−)- $[^{125}\text{I}]$ iodocyanopindolol

(−) isoproterenol (IC_{50} of $7.1 \times 10^{-5}\text{M}$) was 6 times more potent than that of either (−) epinephrine (IC_{50} of $4.0 \times 10^{-4}\text{M}$) or (−) norepinephrine (IC_{50} of $4.0 \times 10^{-4}\text{M}$), indicating the specificity of the $[^{125}\text{I}]$ ICYP binding site (Fig.3). Competition studies with antagonists demonstrate the specificity of the $[^{125}\text{I}]$ ICYP binding site: timolol (IC_{50} of $6.3 \times 10^{-8}\text{M}$) > pindolol (IC_{50} of $2.8 \times 10^{-7}\text{M}$) > metoprolol (IC_{50} of $1.8 \times 10^{-5}\text{M}$) > atenolol (IC_{50} of $2.8 \times 10^{-4}\text{M}$) (Fig.4).

There was a significant negative correlation

Fig. 4. The competition of unlabeled antagonists for $[^{125}\text{I}]$ ICYP binding sites at a ICYP concentration of 200 pM. Left ordinate: Percent inhibition of $[^{125}\text{I}]$ ICYP bound. Abscissa: $-\log$ antagonist concentration in M. $\circ$ =atenolol; $\bullet$ =metoprolol; $\blacktriangle$ =pindolol; $\blacksquare$ =timolol. $[^{125}\text{I}]$ ICYP: (−)- $[^{125}\text{I}]$ iodocyanopindolol

Fig. 5. Correlation between the human right atrial β AR density and age. Left ordinate: Right atrial β AR density (Bmax) in fmol (−)- $[^{125}\text{I}]$ iodocyanopindolol (ICYP) specifically bound/mg protein. Abscissa: Age in year. There was a significant negative correlation between human right atrial β AR density and age ($r=0.575$, $p<0.001$). β AR: β -adrenoceptor

between human right atrial β AR density and age ($r=0.575$, $p<0.001$, Fig.5). Neither MRAP nor CTR was correlated with human right atrial β AR density (Fig.6, 7). Further, there was no significant correlation between human right atrial β AR affinity and age (Fig. 8).

DISCUSSION

In this study, we characterized the β ARs present in human cardiac crude membranes in terms of binding stereospecificity and specificity criteria. Using these assays, we demonstrated age-associated decreases in the

Fig. 6. Correlation between the human right atrial β AR density and MRAP. Left ordinate: Right atrial β AR density (Bmax) in fmol (-)-[125 I]iodocyanopindolol (ICYP) specifically bound/mg protein. Abscissa: MRAP in mmHg. There was no significant correlation between human right atrial β AR density and MRAP. β AR: β -adrenoceptor, MRAP: mean right atrial pressure

Fig. 7. Correlation between the human right atrial β AR density and CTR. Left ordinate: Right atrial β AR density (Bmax) in fmol (-)-[125 I]iodocyanopindolol (ICYP) specifically bound/mg protein. Abscissa: CTR in percent. There was no significant correlation between human right atrial β AR density and CTR. β AR: β -adrenoceptor, CTR: cardiothoracic ratio

number of human right atrial β ARs.

Cardiac β AR responsiveness has been shown to decrease with age¹⁻⁶. In contrast, there are no age-related differences in the chronotropic response to electrical pacing in dogs² or in the positive inotropic response to increasing calcium concentrations in rat

Fig. 8. Correlation between the human right atrial β AR affinity and age. Left ordinate: Right atrial β AR affinity (K_D) in pM. Abscissa: Age in year. There was no significant correlation between human right atrial β AR affinity and age. β AR: β -adrenoceptor

myocardium²⁶). These data suggest that the intrinsic inotropic and chronotropic mechanisms do not change with age. Therefore, the mechanism responsible for the reduced β AR responsiveness in aging involves the interaction between the β AR and its effectors. The β AR system consists of three components: the receptor, the guanine nucleotide-binding regulatory protein (G-protein), and the adenylyl cyclase. With increasing age, each of these components may undergo regulatory changes that result in reduced β AR responsiveness.

Studies of changes in the β AR density with age in humans have been performed mainly using circulating lymphocytes (Table)¹²⁻²⁰. While one of these studies demonstrated that lymphocyte β AR density decreases with age¹², most studies have shown that lymphocyte β AR density does not change¹³⁻¹⁷ or even increases with age¹⁸⁻²⁰. Further, it has been demonstrated by directly comparing lymphocyte and cardiac β AR densities, that changes in lymphocyte β_2 ARs correlate with changes in cardiac β_2 ARs. However, changes in lymphocyte β_2 AR density do not correlate with the density of cardiac β_1 ARs²¹. Because β_1 ARs predominate in the human heart, the ability of lymphocyte β_2 ARs measurements to estimate total cardiac β ARs is limited.

Because it is technically difficult to obtain human cardiac tissue, studies of changes in the cardiac β AR system with age have been performed mainly in rat heart (Table)^{7,8,11,26-32}. While some of these studies have found that the cardiac β AR density decreases with age^{11,27,29,32}, others have demonstrated that the

Table. Age related alteration in β -adrenoceptor density

Reference	Year	Species	Tissue	Ligand	Effect of Aging
Broode et al. ²¹	1995	Human	Heart	[¹²⁵ I] ICYP	→
Schocken et al. ¹²	1977	Human	Lymphocyte	[³ H] DHA	↓
Abrass et al. ¹³	1981	Human	Lymphocyte	[³ H] DHA	→
Landmann et al. ¹⁴	1981	Human	Lymphocyte	[³ H] DHA	→
Doyle et al. ¹⁵	1982	Human	Lymphocyte	[³ H] DHA	→
Broode ¹⁶	1983	Human	Lymphocyte	[¹²⁵ I] ICYP	→
Halper et al. ¹⁷	1984	Human	Lymphocyte	[¹²⁵ I] ICYP	→
Fitzgerald et al. ¹⁸	1984	Human	Lymphocyte	[³ H] DHA	↑
Middeke et al. ¹⁹	1984	Human	Lymphocyte	[¹²⁵ I] ICYP	↑
Zahniser et al. ²⁰	1988	Human	Lymphocyte	[¹²⁵ I] IPIN	↑
Guarnieri et al. ²⁶	1980	Rat	Heart	[³ H] DHA	→
Abrass et al. ⁷	1982	Rat	Heart	[³ H] DHA	→
Narayanan et al. ⁸	1982	Rat	Heart	[³ H] DHA	→
Tumer et al. ³¹	1989	Rat	Heart	[¹²⁵ I] ICYP	→
Kusiak et al. ²⁸	1983	Rat	Heart	[³ H] DHA	↑
Cros et al. ³⁰	1988	Rat	Heart	[¹²⁵ I] ICYP	↑
Baker et al. ²⁷	1980	Rat	Heart	[³ H] DHA	↓
Fan et al. ¹¹	1985	Rat	Heart	[³ H] DHA	↓
Urasawa et al. ²⁹	1986	Rat	Heart	[¹²⁵ I] ICYP	↓
Tanaka et al. ³²	1990	Rat	Heart	[³ H] DHA	↓

Arrows indicate increase (↑), decrease (↓) or no change (→)

[¹²⁵I] ICYP: (-)-[¹²⁵I] iodocyanopindolol, [¹²⁵I] IPIN: (-)-[¹²⁵I] iodopindolol, [³H] DHA: (-)-[³H] dihydroalprenolol

receptor density does not change with age^{7,8,26,31}) or even increases with age^{28,30}). One possible reason for the discrepancy between these studies is the difference in the preparation of myocardial membranes³⁰). Specifically, there are age-related changes in the sedimentation coefficient of the myocardial plasma membrane that may affect its isolation¹¹).

Recently, it has been demonstrated that β AR density does not change in the human right atrial myocardium with increasing age (Table)²²). The authors have concluded that the age-related decrease in human right atrial β AR function was not due to alterations in receptor density but involved an impairment of the activity of the catalytic subunit of adenylyl cyclase. Their conclusion was based on the finding that receptor- and nonreceptor-mediated activation of the enzyme was reduced. Our observations on the effect of aging on human right atrial β AR density are not keeping with their findings. The mean right atrial β AR density in the three age groups in their study (group A, <20 years; group B, 20 to 50 years; group C, >50 years) was not significantly different. Although, there was a tendency toward a decrease in receptor density between the three groups.

Plasma norepinephrine concentrations are known to increase with age^{33,34}). Receptor desensitization in the setting of increased plasma norepinephrine concentrations may contribute to the reduced human right atrial β AR density observed in our study. Because it has been demonstrated that there is no increase in the translocation of receptors from the cell surface to

intracellular sites (internalization) in the aging rat heart³⁵), the mechanism for this desensitization might be degradation of β ARs.

In this study, the β AR density of older patients was similar to previously reported values. In contrast, the β AR density in the younger patients we studied was higher than that previously reported. The cause of this discrepancy remains unclear. However, if the β AR-mediated effects correlate with the total number of β AR, it is possible that the β AR density in younger patients is greater than that of older subjects because the heart weight of younger patients is less than that of older patients. It has been shown that as heart weight increases in the rat, the β AR density per gram of heart decreases during growth²⁷).

Although we did not evaluate the adenylyl cyclase activity in this study, there are several studies which indicate that adenylyl cyclase and/or G-protein activity decreases with age^{6,8~11,28,29,36~38}). Maximum postreceptor activation of adenylyl cyclase by fluoride (which stimulates at the level of the G-proteins) and forskolin (which directly stimulates adenylyl cyclase) decreases with age to the same extent as maximum activation of adenylyl cyclase by isoproterenol^{9,10,36}). These data suggest that the mechanism responsible for the diminished activation of adenylyl cyclase with age is not only receptor desensitization because of increased plasma norepinephrine concentrations with age but also impairment of the postreceptor activation of adenylyl cyclase independent of desensitization^{22,39}).

This study limited by the fact that the effect of

disease on cardiac β AR density cannot be eliminated. However, it has been demonstrated that β AR desensitization relates more to the degree of heart failure rather than to the cause of heart failure⁴⁰. Because all of the patients in this study were without signs of overt heart failure, and neither the MRAP nor CTR correlated with β AR density, we believe that the reduced β AR density relates more to the age of patients rather than to the disease of patients.

In conclusion we found an age-related decrease in human right atrial β AR density. The decrease in human cardiac β AR function with increasing age may be due to the reduced β AR density as well as impairment of adenylyl cyclase activity.

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