Increased Uncoupling of β -, β 1- and β 2-Adrenoceptor to Myocardial Contraction in Failing Human Myocardium

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Coupling of myocardial β -, β 1-, β 2- and α -adrenoceptors (AR) to myocardial contraction was investigated in patients with various degrees of heart failure. With the use of ΔV_{cfc} , a load independent parameter of myocardial contraction, AR mediated contraction was evaluated. β -AR mediated contraction, ΔV_{cfc} by infusion of a β -AR agonist, isoproterenol, declined with the advancement of heart failure from 0.41 Circ/sec (NYHA I) to 0.31 (NYHA II), 0.22 (NYHA III) and 0.12 (NYHA IV). Dobutamine, a β 1-AR full agonist, mediated ΔV_{cfc} was 92–97% of that of isoproterenol. On the other hand, terbutaline sulfate, a full agonist to β 2-AR, increased ΔV_{cfc} partially in comparison with isoproterenol; 51% in NYHA I, 52% in NYHA II, 36% in NYHA III and 17% in NYHA IV. An α 1-AR agonist, methoxamine had little effect on myocardial contractility. β -AR and α -AR densities were analyzed by saturation binding isotherms of myocardial membrane fraction with ^{125}I -Iodocyanopindolol (ICYP) and ^3H -Bunazosin, respectively. β -1 and β 2-ARs were separated by competition binding of ^{125}I -ICYP with a highly selective β 1 AR antagonist, CGP20712A. There was a progressive down regulation of β -, β 1- and β 2-ARs with the advancement of heart failure. A new index was used to examine coupling of ARs to myocardial contraction; Coupling Index. The index was slightly decreased in NYHA II in β - and β 1-ARs. In β 2-AR, the coupling index declined as heart failure advanced from NYHA I to NYHA IV. These results indicate some mechanism(s) of uncoupling takes place in mild heart failure in β and β 1-AR, while in β 2-AR uncoupling progresses from NYHA II to IV. Down regulation of all β -ARs begins from NYHA III onwards.

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THE β -adrenergic system plays the most important role in the modulation of myocardial contractility. To compensate for heart failure, the efferent sympathetic adrenergic nervous system is activated, as

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expressed by elevated circulating nor-epinephrine and epinephrine in patients with heart failure^{1,2}. However, failing human myocardium has been shown to have depressed contraction in response to exogenous β -adrenergic receptor (AR) agonists³ as well as depressed chronotropic and inotropic effects. The depressed cardiotonic response by β -agonist has been at-

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Fig.1. Comparison of the LV end systolic wall stress (σ_{es} , g/cm²) and rate corrected velocity of fiber shortening (Vcfc, Circ/sec), showing method of data analysis. Mean contractility line (MCL) was obtained by linear regression analysis of Vcfc- σ_{es} points before and after intravenous infusion of nitroglycerin (NTG). β -AR mediated contraction, independent of loading conditions, was obtained by measuring vertical distance ($\Delta Vcfc$) from MCL to Vcfc- σ_{es} point after isoproterenol infusion. Afterload reduction contribution was corrected by MCL. Likewise, β 1-AR and β 2-AR mediated contraction parameters were obtained by measuring $\Delta Vcfc$ after infusion of dobutamine and terbutaline, respectively.

tributed to down-regulation of myocardial β -AR⁴; uncoupling of the receptor to effector enzyme⁵ and sequestration of the receptor^{6,7}. Guanine nucleotide binding protein (G protein) is known to be activated by the binding of a hormone or a transmitter to a receptor. Activated G protein modifies effector enzymes; Gs stimulates and Gi inhibits adenylate cyclase, transducin inhibits phosphodiesterase, Gp or Gi modifies phospholipase or ion channels. Recently, G protein abnormality has been found in an experimental model of heart failure and failing human myocardium⁸⁻¹⁰. G Protein abnormality is expressed as uncoupling of receptor number to cellular function. No work has ever been done to compare receptor number with cardiac contraction from mild to end stage of heart failure patients. Biochemical and histochemical studies have shown that human myocardium contains β 2-AR as well as β 1-AR¹¹⁻¹⁵. Understanding of the mechanisms of β -AR subtype regulation is important for decision making in a clinical

situation of treatment in heart failure. The existence of myocardial α 1-AR has been reported, but the function of this receptor has not yet been elucidated!¹⁶⁻¹⁸

This study was undertaken to clarify the abnormalities in receptor number, pure contraction moiety, and receptor-contraction coupling of β -, β 1-, β 2-, and α 1-ARs in various degrees of heart failure.

METHODS

β -, β 1-, β 2- and α 1-Adrenoceptor mediated cardiac contraction

Subjects

Forty eight patients with various degrees of heart failure were enrolled in this study. The experimental protocol was approved by our institutional clinical research committee. All patients gave informed consent, oral or written, to participate in the study. Any inpatient with heart disease of either gender, 20-75 years old of age and able to communicate was included. Patients were

TABLE I

ΔV_{cfc}	NYHA functional class			
	I	II	III	IV
β -Adrenoceptor	0.41	0.31*	0.22*	0.12***
$\beta 1$ -Adrenoceptor	0.38	0.30*	0.21**	0.11***
$\beta 2$ -Adrenoceptor	0.21	0.16*	0.08**	0.02***
$\alpha 1$ -Adrenoceptor	-0.03	-0.02	0.02	0.03

Load independent index of myocardial contractility (ΔV_{cfc} , Circ/sec) by β -, $\beta 1$ -, $\beta 2$ - and $\alpha 1$ -Adrenoceptors in various degrees of heart failure. Mean values, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs NYHA I.

allowed treatment with digoxin, diuretics or angiotensin-converting enzyme inhibitors. Any patient with insulin dependent diabetes mellitus, thyroid diseases, malignant diseases, anemia, receiving treatment with catecholamines, β - or α -blockers was excluded. Patients with extreme obesity or severe lung disease were excluded if carotid arterial pulse was not good or cardiac ultrasound recording was unrecordable. Patients were divided into four groups according to New York Heart Association functional class, NYHA I, II, III and IV. Each group consisted of 12 patients. Mean age was 53, 54, 56 and 58 year old, and proportion of male patients was 67%, 58%, 50% and 50%, in NYHA I, II, III and IV, respectively.

Evaluation of β -, $\beta 1$ -, $\beta 2$ - and $\alpha 1$ -Adrenoceptor mediated cardiac contractility

Pure contraction moiety was analyzed by the method of Borow et al.²⁰⁻²² After intravenous injection of atropin sulfate (1 mg, Tanabe Pharmaceutical Co, Osaka) to block parasympathetic drive, a patient was recorded B mode guided M mode echocardiogram for measurement of left ventricle dimension with carotid arteriogram for blood pressure reference with a patient in left semidecubitus position. Cuff blood pressure was recorded simultaneously from the radial artery. Left ventricular end-systolic wall stress (σ_{es} , g/cm²) was calculated using the following formula¹⁹:

$$\sigma_{es} = \frac{1.35 \times P_{es} \times Des}{4 \times Thes \times (1 + Thes/Des)}$$

where P_{es} is the left ventricular end-systolic

pressure (mmHg), Des and $Thes$ are the left ventricular end-systolic dimension (cm) and posterior wall thickness (cm) and 1.35 is a conversion factor (mmHg to g/cm²). The rate-corrected mean velocity of left ventricular fiber shortening (V_{cfc} , Circ/sec) was calculated as:

$$V_{cfc} = \frac{\% FS}{(ET/(RR)^{1/2})}$$

where % FS is left ventricular percent fractional shortening, RR in the interval between adjacent R waves on the electrocardiogram and ET is the ejection time of left ventricle.

Left ventricular contractile state was assessed with the use of the load-independent relation between left ventricular end-systolic wall stress and rate-corrected velocity of fiber shortening (Fig. 1). For each patient, the baseline relation was determined by linear regression analysis (least-squares methods) of data points obtained under control conditions and during the administration of intravenous nitroglycerin (Nihon Kayaku Co. Ltd., Tokyo, 0.5–2 μ g/kg/min); thus measurements were obtained over a wide range of afterload conditions. For individual values obtained during the infusion of l-isoproterenol hydrochloride (Nikken Kagaku Co. Ltd., Tokyo, 0.005–0.01 μ g/kg/min), dobutamine hydrochloride (Shionogi & Co. Ltd., Osaka, 4–32 μ g/kg/min), terbutaline sulfate (Fujisawa Pharmaceutical Co. Ltd., Osaka, 2–8 μ g/kg/min) or methoxamine hydrochloride (Nihon Shinyaku Co. Ltd., Kyoto, 0.01 mg/kg/min) the vertical distance above this regression line was used to estimate left ventricular contractile response (ΔV_{cfc} , Fig. 1).

Adrenergic Receptor Assay

Subjects

Sixteen patients undergoing mitral valve replacement have been studied for myocardial adrenoceptors assay. Patients were divided into three groups according to New York Heart Association functional class, NYHA II, III and IV. Cardiac index was 2.89 in NYHA II (n=4), 2.53 in NYHA III (n=7, $p < 0.05$ vs NYHA II) and 1.76 l/min/m² in NYHA IV (n=5, $p < 0.01$ vs NYHA II). Left ventricular filling pressure was 9, 18 ($p < 0.01$ vs NYHA II) and 23

Fig.2. Measurement of β , $\beta 1$ - and $\beta 2$ -AR densities of human myocardium.

A: Saturation binding isotherms of human myocardium with ^{125}I -CYP from NYHA II (closed circle) and NYHA IV (open circle) patients.

B: Scatchard plot of ^{125}I -ICYP to human cardiac membranes from patients of NYHA II (closed circle) and NYHA IV (open circle).

C: Competition binding of human myocardial tissue of ^{125}I -ICYP with various concentrations of CGP-20712A. $\beta 1$ -AR was displaced at low concentration of CGP-20712A and $\beta 2$ AR was displaced at higher concentration of CGP-20712A.

TABLE II

<i>B_{max}</i>	<i>NYHA functional class</i>				<i>End Stage</i>
	<i>I</i>	<i>II</i>	<i>III</i>	<i>IV</i>	
β -Adrenoceptor	111	119	69*	34**	28**
$\beta 1$ -Adrenoceptor	69	76	40*	19**	17**
$\beta 2$ -Adrenoceptor	42	43	29*	15**	11**
$\alpha 1$ -Adrenoceptor		21	20	24	

Densities of β -, $\beta 1$ -, $\beta 2$ - and $\alpha 1$ -ARs in various degrees of heart failure.

NYHA II, III and IV were left ventricular myocardium excised at mitral valve replacement.

NYHA I and End Stage heart failure are autopsied heart within 6 hours after death. Mean values, * $p < 0.05$,

** $p < 0.01$, *** $p < 0.001$, vs NYHA I

mmHg ($p < 0.01$ vs NYHA II) in NYHA II, III and IV, respectively. Ejection fraction of left ventricle was 68, 54 ($p < 0.05$ vs NYHA II) and 48% ($p < 0.01$ vs NYHA II) in NYHA II, III and IV, respectively, evaluated by Kennedy's method from left ventriculogram.

Hearts obtained by autopsy within 6 h after death were also included in this study. Seven hearts were from patients with normal functioning heart without catecholamine treatment. Eight hearts were from end stage heart failure (End Stage, NYHA IV), two died of dilated cardiomyopathy, one patient had amyloidosis, one sarcoidosis and other four patients had ischemic cardiomyopathy.

Preparation of Myocardial Sarcolemma

Left ventricular papillary muscle was

minced in 300–500 ml in 4 °C of 25 mM Tris[hydroxymethyl]aminomethane (TRIS)-HCl buffer, pH 7.5 with 250 mM sucrose and 1 mM ethylenediamine tetraacetic acid (EDTA) (SET Buffer) with protease inhibitors (2 $\mu\text{g}/\text{ml}$ leupepsin, 5 $\mu\text{g}/\text{ml}$ soybean trypsin inhibitor and 10^{-4}M phenylmethylsulfonyl fluoride) as soon as possible after removal from the heart. The membrane fraction of the myocardium was prepared according to the method as described below. All procedures were performed at 4 °C unless otherwise noted. Papillary muscle was minced with a razor blade into small pieces. The minced tissue was cut into smaller pieces with a Waring blender after they were mixed with 5–10 times volumes of SET buffer with protease inhibitors. The mixed tissue was

TABLE III

Coupling Index	NYHA functional class			
	I	II	III	IV
β -Adrenoceptor	3.69	2.61	3.19	3.52
β 1-Adrenoceptor	5.51	3.95	5.25	5.79
β 2-Adrenoceptor	5.0	3.72	2.76	1.33
α 1-Adrenoceptor		-0.95	1	1.25

Adrenoceptor to Myocardial Coupling Index of β -, β 1-, β 2- and α 1-ARs in various degrees of heart failure.

then homogenized with Polytron (Brinkmann Instruments, Westbury, NY, USA) for 3 bursts of 10 sec. The crude homogenate was centrifuged for 15 min at 1,000g. The supernatant was filtered through 3 layers of gauze and the filtrate was transferred to ultracentrifuge tubes. After centrifugation for 30 min at 100,000g, crude membrane fraction was obtained as a precipitate. The membrane fraction was washed twice with 75 mM TRIS-HCl buffer pH 7.5 with 1 mM EDGA and 10 mM MgCl₂ with protease inhibitors. The membrane fraction was stored at -80 °C until use. Protein was analyzed by BCA reagent with bovine serum albumin as a standard²³

β -, β 1-, β 2- and α 1-Adrenoceptor Density Analysis

β -Adrenoceptor density was analyzed by saturation binding isotherm with (-)-¹²⁵I-Iodocyanopindolol (ICYP, New England Nuclear, Boston U.S.A., specific activity 81.4 TBq/mmol). Membrane (3–10 μ g/tube) was incubated with 75 mM TRIS-HCl buffer pH 7.5, 1 mM EDGA, 10 mM MgCl₂, 10⁻⁴M GTP, 10⁻⁴M ascorbic acid and 0–120 pM ¹²⁵I-ICYP. Nonspecific binding was determined in the presence to 10⁻⁴M l-isoproterenol. After incubation of 120 min at 37 °C, reaction was terminated by the addition of 3.5 ml of ice cold 75 mM TRIS-HCl buffer pH 7.5 with 1 mM EDGA and 10 mM MgCl₂. The mixture was transferred over Whatman GF/C filter paper. The filter was washed two more times with 10 ml of the same buffer. The filter paper was dried and radioactivity was counted by a gamma-counter (Packard 2200ILC, Packard, USA). All samples were counted for triplicates.

Specific binding was calculated by subtracting nonspecific binding from total binding. Nonspecific binding was 9–12% at the K_d value of ICYP in normal to mild heart failure and 15–20% in moderate to severe failing myocardium. Densities of β -adrenoceptor (B_{max}) and dissociation constant (K_d) was obtained by Scatchard analysis²⁴ with least square methods using desktop microcomputer using LIGAND²⁵ as shown in Fig. 2B. α 1-Adrenoceptor density was assayed with ³H-bunazosin HCl (a kind gift of Dr. Miyagi at Eisai Pharmaceutical Co. Ltd., Amersham, England, specific activity 2.00 TBq/mmol) by saturation binding isotherm. Incubation buffer was the same as of β adrenoceptor assay but MgCl₂ was omitted and nonspecific binding was a binding in the presence of 10⁻⁴M phentolamine. β 1 and β 2 adrenoceptor was analyzed by competition binding isotherm of ICYP with a highly specific β 1 antagonist, CGP-20712A (Ciba-Geigy, Basle, Switzerland). Aliquots of membrane was incubated with buffers used in total binding in β -AR, with a different concentrations of CGP-20712A from 10⁻¹¹ to 2 $\times$ 10⁻³M. As shown in Fig. 2C, clear discrimination of β 1-AR and β 2-AR was obtained. Densities of β -AR subtypes (β 1- and β 2-ARs) and dissociation constants were obtained by LIGAND program²⁵

Myocardial Contraction per Single Receptor

A new index, coupling index was invented to express myocardial contraction per single receptor.

$$\text{Coupling Index (C. I.)} = \frac{\Delta V_{cfc}}{B_{max}}$$

This index represents a unit myocardial contraction per single receptor; the coupling of a receptor to myocardial contraction in each stage of heart failure.

Statistical Analysis

Statistical analysis was performed by Student's *t*-test. Statistical significance was attained if *p* < 0.05. Linear regression was calculated by a least squares method with a microcomputer. LIGAND²⁵ was used to calculate parameters from Scatchard plot and competition analysis with CGP-20712A for two binding sites fitting.

MATERIALS

Radioisotope used in this study were ^{125}I -Iodocyanopindolol (specific activity, 81.4 TBq/mmol) from New England Nuclear and a kind gift from Eisai Pharmaceutical Co. Ltd., ^3H -Bunazosin (Amersham, England, specific activity 2.00 TBq/mmol). For contraction experiments, atropin sulfate (Tanabe Pharmaceutical Co, Osaka), nitroglycerin (Nihon Kayaku Co. Ltd., Tokyo), dobutamine HCl (Shionogi & P Co. Ltd., Osaka), terbutaline sulfate (Fujisawa Pharmaceutical Co. Ltd., Osaka) and isoproterenol (Nikken Kagaku Co. Ltd, Tokyo) were used. CGP-20712A was a kind gift from Dr. H. Schroter and Dr. K. Scheibli of Ciba-Geigy, Ltd, Basel, Switzerland. Tris[hydroxymethyl]aminomethane, HCl, sucrose, ethylenediaminetetraacetic acid, leupepsin, soybean trypsin inhibitor and phenylmethylsulfonyl fluoride, and MgCl_2 were purchased from Sigma chemical company, St. Louis, MO, U.S.A. All other reagents were of highest grade available.

RESULTS

Contraction study

All patients were stable in blood pressure and mental condition during recording carotid arteriogram and B mode guided M mode echocardiogram before and during intravenous infusion of nitroglycerin, isoproterenol, terbutaline, dobutamine and methoxamine. Basal percentage fractional shortening (% FS) was 32%, 25%, 16% ($p < 0.01$ vs NYHA I), and 12% ($p < 0.01$ vs NYHA I) and an index of afterload, end-systolic wall stress, calculated by the method of Brodie BR et al,¹⁹ was 62, 72 ($p < 0.05$ vs NYHA I), 108 ($p < 0.01$ vs NYHA I) and 126 g/cm^2 ($p < 0.01$ vs NYHA I) in NYHA I, II, III and IV, respectively. After depicting V_{cfc} vs σ es of control and during four to five points of nitroglycerin infusion, a mean contractility line could be obtained (Fig. 1). β -AR mediated contractility, as expressed by ΔV_{cfc} (Circ/sec) by isoproterenol, declined as heart failure advanced (Table I). Statistical significance was apparent from NYHA II.

Activation of myocardial contractility through β -1 and β -2 ARs was examined by

infusion of dobutamine and terbutaline, respectively. Dobutamine, at least a full agonist to β 1-AR, acted as a full agonist of β - and β -1 adrenoceptors from mild to severe states of heart failure. On the other hand, terbutaline sulfate, a full agonist of β 2 AR, activated β AR partially (Table I). The partiality of terbutaline for β AR decreased as heart failure advanced from 51.2% in NYHA I to 16.7% in NYHA IV.

Measurements of Adrenergic Receptors

Densities of β , β 1 and β 2-ARs were examined in human myocardium of various degrees of heart failure. As shown on Fig. 2A, human myocardium showed saturable binding with increasing concentrations of ^{125}I -ICYP, indicating saturable radioligand binding site or receptor. Scatchard plot was linear as shown on Fig. 2B, indicating a single binding site. Hill coefficient was 0.9 to 1.0, confirming one radioligand to one receptor binding site. Densities of the receptor (B_{max}) and dissociation constant (K_d) were obtained by linear regression analysis from the Scatchard analysis. Densities of β AR decreased as heart failure advanced from NYHA I to NYHA IV as shown on Table 2. There was no significant difference in β -AR number between NYHA I and NYHA II, statistical significance was apparent in NYHA III and NYHA IV compared with NYHA I. A highly selective β 1-AR antagonist, CGP-20712A, sharply discriminated β 1 and β 2-ARs as shown on Fig. 2C with pK_d values of 8.90 for β 1 and 5.61 for β 2-ARs. There was no significant differences in pK_d values between normal and end stage heart failure. As summarized in Table II, densities of β 1 adrenoceptor decreased as heart failure advanced. Likewise, densities of β 2-AR declined with advancement of heart failure. Percentile changes of β 1-AR in total β -AR was 62.2% in NYHA I, 63.9% in NYHA II, 58.0% in NYHA III, 55.9% in NYHA IV and 60.7% in end stage heart failure. Densities of α 1-AR was measured by saturation binding isotherms of myocardial membrane with ^3H -bunazosin. The binding was saturable with nonspecific binding 30–50% in concentration near K_d of ^3H -bunazosin. There was no significant difference in densities of α 1-AR from NYHA II to NYHA IV human myocardium,

as indicated in Table II.

Coupling index was calculated from mild to severe heart failure according to NYHA functional class and results are summarized on Table III. In β -AR, coupling index declined by 29.3% in NYHA II, by 13.6% in NYHA III and by 4.6% in NYHA IV compared with NYHA I. Similarly, in β 1-AR, coupling index decreased by 28.3% in NYHA II, by 4.7% in NYHA III and increased by 5.1% in NYHA IV compared with NYHA I. On the other hand, coupling index decreased as heart failure advanced in β 2-AR. Coupling index decreased by 25.6% in NYHA II, 44.8% in NYHA III and by 73.4% in NYHA IV compared with NYHA I.

DISCUSSION

Tachyphylaxis to β agonists is a commonly observed phenomenon in failing human myocardium.³ By the lack of a good clinical index, quantitative evaluation of myocardial contraction has been a difficult task. With the use of a pure index of myocardial contractility, ΔV_{cfc} , we have demonstrated depressed contraction by β , β 1 and β 2 AR in failing human myocardium, from mild to severe heart failure (Fig. 2 and Table I). Bristow et al^{5,11} compared activation of β -AR subtype in accordance with β -AR subtype measurement in failing heart. Isolated right ventricular myocardial tissue of dilated cardiomyopathy exhibited denopamine (a β 1-selective and partial β 1-agonist²⁶) specific tachyphylaxis in contraction as well as in cyclic AMP production. Myocardial contraction and c-AMP production by zinterol, a β 2 agonist was relatively spared. Brodde et al¹²⁻¹⁵ reported nonselective tachyphylaxis in myocardial tissue from patients with mitral valve disease.

Elevated synaptic norepinephrine in heart failure can be responsible for agonist induced tachyphylaxis. Norepinephrine is a β 1 selective agonist as well as nonselective α -AR agonist. If agonist specific desensitization developed, selective down regulation of β 1 and α 1-ARs should have occurred. β 1-AR selective down regulation have been reported in hearts of dilated cardiomyopathy or aortic valve disease. Our results were a down regulation of both β 1 and β 2-ARs,

which is agonist nonspecific down regulation. The difference remains unclarified, but disease specificity has been reported. Brodde et al¹⁴ reported similar results as in this study in mitral valve diseases. Our results support their disease specific β -AR subtype down regulation hypothesis.

The presence of myocardial α 1-AR has been reported in normal and failing human myocardium. The receptor has been proposed to be involved in myocardial contraction,²⁸ cell growth²⁹ and myocardial ischemia.³⁰ We could not elucidate any functional or pathological role of α -AR in this study. Colan et al used methoxamine as an afterload increasing agent, not a inotropic agent,²¹ which is relevant to our results. Species specificity seems important in this receptor. Thus, the function of the receptor remains unclarified. Myocardial contraction by α 1-AR was weak and not altered by the advancement of heart failure.

Several reports have suggested an increased uncoupling of α -AR to adenylate cyclase in failing human myocardium.^{5,8,9} Receptor binding studies showed enhanced GTP shift in failing human heart with the shift to be weak, indicating an uncoupling of the receptor to G protein while coupling of α -AR to G proteins is tight.³¹ Abnormal densities of G proteins have been reported in experimental heart failure⁸ and failing human myocardium.⁹ Uncoupling of β 2-AR was reported by Bristow et al⁵ where densities of β 2-AR was similar while adenylate cyclase activity response was depressed. Our results are increased uncoupling of β 2-AR to myocardial contraction, where β 2-AR was down regulated and more depressed myocardial contraction by the receptor.

There are some contradictions between our results and previously reported ones. The differences of race; results may be different in Mongoloid and Caucasian populations. Orientals have been reported to be very sensitive to even a small amount of β -blockers. Mechanisms of β -AR subtype specific down regulation have been reported different based of underlying myocardial diseases. Our results demonstrate that receptor-contraction coupling differs in various degrees of heart failure. Methods of evaluation are critical point in clinical situation. Recently, pressure-volume relation

have been established to give us load independent parameters, $E_{\max}$ and E_a .²⁷ But, these indexes have been reported to depend on heart size, which can make group specific evaluation difficult. Another problem is pharmacological agents to be used. There is no disagreement to call isoproterenol to be a full agonist to β , β_1 - and β_2 -ARs. Dobutamine is a full agonist to β_1 -AR, but has some β_2 and α_1 -AR activity. Specificity of denopamine has been established but differences between cAMP increment and positive inotropic effects needs clarified.²⁶ Development of selective agonists and antagonists on clinical use is waited.

Pharmacological,¹⁴ biochemical⁵ and molecular biological³² studies have revealed β -adrenergic pathway: β -adrenoceptor, G protein, adenylyl cyclase, A kinase, substrates of A kinase, contractile components, phospholamban, calcium channels and so on. β -Blockers have been extensively used in many clinical situations. Cardiologist should make best use of these tools of basic science for the best use of various β -agonists and antagonists for the treatment of heart diseases including congestive heart failure, angina pectoris, myocardial infarction, arrhythmia, hypertension, hypertrophic cardiomyopathy.

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