Regulation of the Catecholamine β-Adrenergic System in Ventricular Remodeling of Hypertension

Peng YINGXIN,1 PhD, Shan JIANG,1 MD, Qi XIAOYONG,2 MD, Xue HAO,2 MD, Rong CHUNLI,2 MD, Yao DONGMEI,2 MD, Guo ZHIQIN,2 MD, Zheng SHILING,2 MD, and Wu MIN,3 MD

SUMMARY

Differences in structural remodeling are believed to be influenced by hormonal systems in hypertension. The objective of the present study was to investigate the change in the circulating catecholamine β-adrenergic system in the left ventricle remodeling process in hypertensives.

One hundred and thirty-four men (mean age, 53 years) had essential hypertension and underwent echocardiography before treatment. Normal morphology (n = 26) and concentric remodeling (n = 41) were defined by a relative wall thickness at diastole (RWT) of < 0.44 and ≥ 0.44, respectively, and concentric hypertrophy (n = 28) and eccentric hypertrophy (n = 39) by a left ventricular mass index (LVMI) of < 150 g/m² and ≥ 150 g/m², respectively. Forty healthy males were studied as normal controls. Plasma levels of norepinephrine (NE) and epinephrine (E) were measured by high performance liquid chromatography. The density of lymphocyte β-adrenoceptors (β-AR) and the content of intralymphocyte cyclic AMP (cAMP) in peripheral blood were measured using ³H-dihydroalpneol as a ligand and protein binding assay, respectively.

The plasma levels of NE and E in the 4 groups of patients with essential hypertension were significantly increased compared with the control group. The density of lymphocyte β-AR and the content of intralymphocyte cAMP of peripheral blood in the normal morphology, concentric remodeling, and concentric hypertrophy groups were significantly higher than those in the control group, while the values in the eccentric hypertrophy group were significantly lower than those in the control group. Among the 4 groups, the plasma levels of NE and E had increased the most in the normal morphology group, followed in decreasing order by the concentric remodeling, concentric hypertrophy, and eccentric hypertrophy groups; the density of lymphocyte β-AR and the content of intralymphocyte cAMP of peripheral blood in the normal morphology, concentric remodeling, and eccentric hypertrophy groups increased while they decreased in the eccentric hypertrophy group in patients with essential hypertension.

The catecholamine β-adrenergic system appears to be related to left ventricular remodeling of hypertension. In this process, catecholamines increased continually. The
density of β-AR and the content of cAMP in peripheral lymphocytes increased at first and then decreased. (Jpn Heart J 2004; 45: 285-296)

**Key words:** Hypertension, Ventricular remodeling, Catecholamine, β-Adrenoceptor, Cyclic AMP

Left ventricular remodeling has been found to be an independent risk factor associated with a marked increased risk for cardiovascular morbidity and mortality. Accordingly, it is worthwhile to study the mechanism(s) of left ventricular remodeling. Recent studies in patients with hypertension have shown that the sympathetic nervous system, especially through β-adrenoceptor (β-AR) activation, appears to be of major importance in the genesis or progression of cardiac remodeling. Available evidence shows increases in the plasma catecholamine concentration, the upregulation of the density of β-AR in myocardium and blood lymphocytes, and increases in mRNA expression in cardiac β-adrenergic signaling in the spontaneously hypertensive rat and myocardial hypertrophy in vivo. Although neurohormonal activation has been suggested as a mechanism for this inappropriate ventricular remodeling, the exact mechanism of regulation of plasma catecholamines in different ventricular morphologies of hypertension present remains to be delineated. Regulation of the β-adrenergic pathway in particular is extremely important since it mediates the biological effects of plasma catecholamine on myocardial cells. Nevertheless, the characteristics of the β-adrenergic pathway in the left ventricular remodeling process have not been previously reported. We therefore sought to clarify the influence of the β-adrenergic pathway in the left ventricle remodeling process in hypertensives.

**METHODS**

**Patients:** Subjects enrolled in this study were adults who were followed up at the Cardiac Center of Hebei Provincial People's Hospital, Shijiazhuang, with a history of hypertension and classified according to the 6th Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC VI). There were 55 males in class 1 and 79 males in class 2. Left ventricular echocardiograms were obtained in all consenting patients. The patients were only admitted to the study if left ventricular echocardiograms of adequate technical quality could be obtained. Their average age was 53 years (range 24 to 68), average body mass index was 22.9 kg/m² (range 16 to 31), and left ventricular ejection fraction was more than 60%. Exclusion criteria included an age greater than 70 years, secondary hypertension, an endocrine or metabolic disease, and
other chronic diseases. None of the patients had taken any sympathomimetic or parasympathomimetic medicine within one month before the study. Forty male subjects were also studied as controls in the same term (average age, 56.2, range, 26 to 68 years) average body mass index 22.7 (range, 20 to 34) kg/m², and no subject had a history of chronic disease and none had taken any medicine with one month before the study. There were no significant differences in age and body mass index between the EH group and control group (P > 0.05). All studies were carried out after an appropriate washout of all cardiac medication and after written informed consent was obtained from every patient before study entry.

**Echocardiography:** Comprehensive 2-dimensional Doppler echocardiography (Toshiba 6000, 2.5 MHz, Japan) was performed as described previously before measurement of plasma levels of norepinephrine and epinephrine, the density of lymphocytes β-AR, and the content of intralymphocyte cAMP. Left ventricular mass (LVM) was determined from M-mode measurements by use of the cube formula according to the recommendations of the American Society of Echocardiography (ASE).¹¹ This formula can easily be transformed to reflect anatomic measurements: \( LVM = 0.80 \times (ASE \text{ mass}) + 0.6 \text{ g} \).¹² LVM was divided by body surface area to obtain the left ventricular mass index (LVMI). Left ventricular hypertrophy (LVH) was defined as an LVMI > 150 g/m², according to data from the Framingham Heart Study.¹³ A partition value of 0.44 was used for relative wall thickness (RWT) \([\text{RWT} = (\text{IVS}+\text{PW}) / \text{LVEDD}, \text{where IVS is the interventricular septum, PW the posterior wall, and LVEDD is the left ventricular end-diastolic diameter}]\).¹⁴ Thus, left ventricular geometry was considered normal if RWT was < 0.44 and LVMI was < 150 g/m². A normal LVMI with increased RWT was denoted concentric remodeling,¹⁴ and a hypertrophy was denoted concentric if the RWT increased and eccentric if the RWT was normal. Examination and analysis of the images were conducted by an experienced physician (Dr JH Wang) who was unaware of other data for the subjects.

**Measurement of the density of β-adrenoceptors in peripheral lymphocytes:** Measurements were taken in the morning with the subjects on an empty stomach, motionless, and silent. Refer to the radioligand binding established by the Chinese Military Medicine Academy. Blood samples (8 mL heparinized for measuring β-AR density) were taken at 8 AM when the patients had rested 30 minutes in a sitting position, preserved in ice water within one hour, isolated, washed, and identified. Lymphocytes were prepared by the method of Shan.¹⁵ The supernatant (50 µL) containing lymphocytes was obtained and adjusted to 6-8 × 10⁶ cells/mL for the radioligand binding reaction in a total volume of 250 µL Tris-HCl to determine the β-AR density with ³H-dihydroalpneol (³H-DHA) at a final concentration 0.8-0.4 nmol/L (specific binding) and propranolol as a nonselective β-blocker (nonspecific binding), respectively. The highest degree of binding sites (the max-
imal number of determining β-AR, B_{max} and the equilibrium dissociation constant (K_D) for DHA were calculated by the formula of Scatchard.

Measurement of plasma levels of norepinephrine (NE) and epinephrine (E): The plasma levels of NE were measured by high performance liquid chromatography.

Measurement of intralymphocyte cyclic AMP (cAMP) content: The intralymphocyte cAMP content was measured by radioimmunoassay as follows. Lymphocytes were isolated and the supernatant containing lymphocytes was agitated by cell ultrasonication (60 W, 15 seconds). All cells were observed with shaking under a microscope, removed, and in by anhydrous alcohol and dried in a 60°C water bath. Reagent boxes of cAMP came from the Institute of Chinese Nuclear Energy. Relative activity of ³H-cAMP was beyond 740 Gbqmmol/L (radioactivity 98%). Determination was performed according to the manufacturer's protocol.

Statistical analysis: The clinical data from the EH patients, including body mass index, heart rate, systolic and diastolic blood pressure, left atrial diameter, posterior wall thickness, diastolic dimension, relative wall thickness, left ventricular mass index, stroke index, cardiac index, total peripheral resistance, plasma levels of NE and E, lymphocyte B_{max}, K_D, and intralymphocyte cAMP content are presented as the mean ± SEM. The differences between the LV morphology group and control group were analyzed by the unpaired Student's t test. Differences in the plasma levels of NE and E, lymphocyte B_{max}, and intralymphocyte cAMP content according to LV morphology were tested by ANOVA. Statistical significance was assumed at P < 0.05. All analyses were performed using Statistical Package for Social Sciences software (SPSS 10.0 for Windows, SPSS Inc, Illinois) and an IBM-PC/AT computer.

RESULTS

Clinical characteristics: Comparisons among the LV geometry groups with respect to various independent variables are presented in Table 1. The groups with concentric remodeling and concentric hypertrophy appeared to have a significantly higher heart rate than the other groups, although the differences were not statistically significant. The concentric hypertrophy and eccentric had the highest stroke index, cardiac index, and total peripheral resistance. There were no differences in the concentrations of creatinine or plasma potassium and sodium.

Results of measurement of plasma levels of catecholamines: Plasma levels of NE and E in the normal morphology, concentric remodeling, concentric hypertrophy, and eccentric hypertrophy groups were significantly increasing compared with the control group. Plasma levels of NZ and Z were the highest in eccentric hypertrophy group, followed in order by the concentric hypertrophy, concentric remodeling and normal morphology groups (Figure 1).
Changes in density of β-adrenoceptors in peripheral lymphocytes: The $B_{\text{max}}$ of peripheral lymphocytes in the normal morphology, concentric remodeling, and concentric hypertrophy groups were significantly up-regulated compared to the
control group, while it was significantly down-regulated in the eccentric hypertrophy group compared to the control group (Figure 2). The $B_{\text{max}}$ of peripheral lymphocytes was the highest in concentric hypertrophy group, followed in order by the concentric remodeling and normal morphology groups. The $K_D$ in patients with EH and in the control group were not significantly different.

**Changes in intralymphocyte cyclic AMP content:** The intralymphocyte cAMP levels of peripheral blood in the normal morphology, concentric remodeling, and concentric hypertrophy groups were significantly higher and significantly lower in the eccentric hypertrophy group compared to the control group (Figure 3). The intralymphocyte cAMP level in peripheral blood was the highest in the concentric hypertrophy group, followed in order by the concentric remodeling and normal morphology groups.

![Figure 2. Changes in the density of lymphocyte β-adrenoceptors in 4 morphology groups. ANOVA test was used to assess the differences. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs control group; $\delta P < 0.05$, $\delta\delta P < 0.01$, $\delta\delta\delta P < 0.001$ vs normal morphology group; $\tilde{P} < 0.05$, $\tilde{\delta} P < 0.01$ vs concentric remodeling group; $\ddot{P} < 0.001$ vs concentric hypertrophy groups.](image1)

![Figure 3. Changes in intralymphocyte cAMP content in 4 morphology groups. ANOVA test was used to assess the differences. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs control group; $\hat{P} < 0.05$, $\hat{\delta} P < 0.01$, $\hat{\delta\delta} P < 0.001$ vs normal morphology.](image2)
DISCUSSION

The present study provides 2 new major findings. First, plasma levels of NE and E were significantly increased, in ascending order, in the normal morphology, concentric remodeling, concentric hypertrophy, and eccentric hypertrophy groups. Second, the density of lymphocyte β-AR and the content of intralymphocyte cAMP in peripheral blood also increased significantly, in ascending order, in the normal morphology, concentric remodeling, and concentric hypertrophy groups, but was significantly decreased in the eccentric hypertrophy group.

Experimental findings have suggested that catecholamines increase protein synthesis and play a role in cardiac hypertrophy.16,17) Raised plasma catecholamine levels have more specifically been related to an increased interventricular septum thickness.16) Schroeder, et al18) found a statistically negative correlation existed between catecholamines and the end-diastolic left ventricular internal diameter index. Further evidence that the sympathetic nervous system could affect left ventricular geometry comes from experimental studies in dogs in which repeated pressor episodes with elevated plasma norepinephrine levels9) or chronic infusion of norepinephrine19) resulted in myocardial hypertrophy but did not induce a sustained elevation of blood pressure. The heart increases its muscle mass and changes its morphology in response to increased catecholamine stimulation resulting from physiologic or pathologic states. Although this response is initially compensatory, it may progress to a pathologic state.

The catecholamine/β-adrenergic receptor (β-AR)/ cyclic AMP (cAMP) axis is the central signaling pathway that serves to stimulate cardiac function.6,20) It is classically perceived as a linear signaling cascade and the key for stimulation of the cascade is an accumulation of the secondary messenger cAMP, which is thought to be responsible for the positive inotropic and chronotropic effects of catecholamines.21) Previous studies have shown that the density of β-AR and the content of cAMP in peripheral lymphocytes were significantly positively correlated with these in myocardial,22,23) accordingly, the quantitative changes in β-AR and cAMP in peripheral lymphocytes served as indirect indicators of the quantitative changes in β-AR and cAMP in the myocardium.24-26) Consequently, we consider, our findings in the process of ventricular remodeling, (the changes of β-AR and cAMP in peripheral lymphocyte) can also reflect local changes of myocardium.23) Increases in systemic and local myocardial sympathetic activity with the resulting release of the endogenous sympathetic hormones epinephrine and norepinephrine activate cardiomyocyte β-AR, which, although responsive to the same hormonal ligands, stimulate almost entirely distinct signaling pathways with different end organs.20,27) Previous studies have shown that the stimulation of chronic sustained intrinsic sympathomimetic-like actions on β-AR of cardiomyo-
ocytes, gives rise to alterations in cardiac β-adrenergic signaling\(^28-30\)) and induces myocardial hypertrophy.\(^31,32\)) These studies are also consistent with our previous studies concerning the relationship between left ventricular mass index and β-AR density in peripheral lymphocytes in patients with HE,\(^33\) and another study concerning changes in β-AR at different stages of hypertension.\(^34\)

An important finding of this study was that plasma levels of NE and E were significantly increased in the concentric remodeling, concentric hypertrophy, and eccentric hypertrophy groups, and these changes were simultaneous with marked increases in stroke index, cardiac index, and total peripheral resistance (Table), suggesting that the normal morphology, concentric remodeling, concentric hypertrophy, and eccentric hypertrophy groups were in a different developmental stage of LV remodeling and that catecholamines are continually increasing in this process. In addition, our finding that β-AR and cAMP pathway activation is characteristic of up-regulating first and down-regulating later, although there is no direct cell pathology evidence to support this hypothesis in the present study. It is possible that geometric patterns with normal morphology, concentric remodeling, and concentric hypertrophy are a series of process for appropriate or super-compensating stage of cardiovascular system, whereas eccentric hypertrophy was a start of inappropriate stage of cardiovascular system. Studies have demonstrated in early stages of hypertrophy that activation of the catecholamine-β-adrenergic system activates a series of subclinical pathophysiological processes (the expression is cardiac remodeling).\(^35,36\)) Other studies have showed that at the compensatory stage of hypertension, β\(_1\)-AR density and responsiveness related to an increment in the plasma catecholamine concentration were increased, whereas they were decreased due to desensitization towards the target hormone in the circulation along with sustained activation of neurohormone.\(^27,36\)

The other important finding of this study was before some LV remodeling phenomena, ventricular hypertrophy and dilatation, have not appeared in clinical, the density of lymphocytes β-AR and the content of intralymphocyte cAMP in peripheral blood have significantly increased such as normal morphology group (RWT and LVMI are normal); and before heart failure haven't appeared in clinical, the density of lymphocytes β-AR and the content of intralymphocyte cAMP in peripheral blood have significantly decreased. This early variation in subcellular level suggests a possible mechanism by which changes in the intracellular biochemical message transduction system are earlier than the changes in the pathologic structural changes in LV remodeling and myocardial function.\(^23\)

Among the leading factors thought to adversely influence ventricular hypertrophy are adrenergic and renin-angiotensin stimulation in ventricular hypertrophy. Recent studies have revealed the mechanisms for this interaction and cross-talk between the systems. An adverse consequence of primary importance from
renin-angiotensin is angiotensin II (Ang II), with plasma and myocardial levels often rising later in the evolution of ventricular hypertrophy. Angiotensin II has both direct and sympathostimulatory effects on the myocardium. Direct effects in normal tissue include hypertrophic signaling.\textsuperscript{37-40} The present study demonstrates that sympathostimulation is central in modulating the Ang II synergy. Sympathostimulatory effects stem from presynaptic and postsynaptic modulation of norepinephrine (NE) and baroreflex modulation.\textsuperscript{41-44} This pathway may also be important because previous studies have shown that Ang II-mediated myocardial tissue damage in rats is inhibited by propranolol,\textsuperscript{45,46} and experimental models of hypertension have also reported that the ability of high-dose but not low-dose atenolol to inhibit Ang II synergy may have been related to incomplete blockade by the latter and/or to loss of $\beta_1$ versus $\beta_2$ selectivity and thus more comprehensive antagonism with the higher dose.\textsuperscript{47,48} Hideaki, et al\textsuperscript{49} reported that marked exacerbation of diastolic dysfunction induced by Ang II infusion was prevented by $\beta$-receptor blockade.

One limitation of the present study is that the number of patients was small. In addition, the information concerning genes with altered expression in the course of ventricular remodeling was not obtained. A few studies have examined gene expression changes during cardiac hypertrophy such as myosin heavy chain,\textsuperscript{50} and analysis of gene expression during the induction of cardiac hypertrophy has revealed a specific transcriptional pattern associated with this process.\textsuperscript{51} Early mediators of the hypertrophic transcriptional program include the immediate-early genes (eg, c-fos, c-myc, and c-jun), followed by a cascade of mitogen-activated protein kinases.\textsuperscript{52,53} These changes contribute to substantial alteration in the expression and organization of sarcomeric and structural proteins.\textsuperscript{54-56} In addition, further follow-up to evaluate the effects of the above changes in the catecholamine-$\beta$-adrenergic system on clinical outcome in the LV remodeling process is planned.

In conclusion, the catecholamine-$\beta$-adrenergic system takes part in the process of LV remodeling of hypertension. In this process, catecholamines increased continually. The density of $\beta$-AR and the content of cAMP in peripheral lymphocytes first increased and then decreased. The changes in the intracellular biochemical message transduction system occur earlier than the pathologic structural changes of LV remodeling and myocardial function.

REFERENCES


38. Watanabe AM, Endoh M. Relationship between the increase in Ca²⁺ transient and contractile force induced by angiotensin II in aequorin-loaded rabbit ventricular myocardium. Cardiovasc Res 1998; 37: 524-31


