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Effects of diltiazem on down-regulation of lymphocyte β-adrenoceptors in patients with chronic congestive heart failure[1]

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KEY WORDS bata-adrenergic receptors; diltiazem; calcium; norepinephrine; congestive heart failure

ABSTRACT

AIM: To determine whether diltiazem could reverse down-regulation of lymphocyte β-adrenoceptor (β-AR) in patients with chronic congestive heart failure (CHF). **METHODS**: Before and after the treatment with diltiazem in CHF patients, lymphocyte β-AR density was measured with [^{3}H]dihydroalprenolol radioligand binding assay, levels of free cytosolic calcium ($[Ca^{2+}]_i$) in platelets were estimated with fluorescent indicator Fura 2-AM, plasma norepinephrine (NE) levels were measured with ^{125}I-radioimmunoassay. **RESULTS**: Lymphocyte β-AR density was lower and $[Ca^{2+}]_i$ in platelets was significantly higher in CHF patients than those in control. Plasma NE levels were higher in CHF patients than those in control. Diltiazem therapy reduced $[Ca^{2+}]_i$ in platelets and increased lymphocyte β-AR density in CHF patients without significant change of plasma NE concentration. **CONCLUSIONS**: Diltiazem partly reversed down-regulation of lymphocyte β-AR density in CHF patients, and this effect was not related to the level of plasma NE, and might be attributed to intracellular $[Ca^{2+}]_i$ decrease.

INTRODUCTION

Since Covell[1] found the reduced cardic response to postganglionic sympathetic nerve stimulation in heart failure in 1966, many studies[2,3] have demonstrated down-regulation of β-adrenoceptor (β-AR) in cardiomyocytes in animal models and patients with CHF, but the mechanism of which is incompletely demonstrated yet. Some researches[4-6] have found that there was abnormal intracellular calcium handling in cardiomyocytes in animal models and patients with CHF. An inverse relation also appears to exist between β-AR number and the concentration of norepinephrine (NE) in blood plasma of heart failure patients[7,8]. However, the role of increased $[Ca^{2+}]_i$ or NE in causing β-AR down-regulation is not established. Calcium-channel blockers are useful in the treatment of several cardiovascular disorders. Hedberg *et al*[9] reported a 46 % (65 % increase in β-AR density in human atria treated with the calcium antagonist verapamil. Yonemochi *et al*[10] observed that β-AR density of cultured rat ventricular myocytes increased by 45 % after 24-h incubation with verapamil 1 mmol/L. It has been proposed that β-AR density increase caused by calcium channel antagonists was partly due to a decrease in $[Ca^{2+}]_i$[11]. We have recently demonstrated that verapamil could increase β-adrenoceptor density and inhibited NE-induced β-adrenoceptor down-regulation in cultured cardiomyocytes *in vivo*[12]. The purpose of present study is to determine whether the calcium antagonist can increase lymphocyte β-adrenoceptor density in CHF patients, and to explore the possible mechanism (s) of this effect.

MATERIALS AND METHODS

Patients Patients (10 men and 7 women; average age, 56 a ± 5 a) with CHF (New York Heart Association Class Ⅱ, Ⅲ) were randomly admitted to the study. Patients (6 men and 4 women; average age, 57 a ± 6 a, group A) were treated with diltiazem (30 mg, tid, *po*) for 14 d despite previous therapy with digitalis and diuretics. The other patients (4 men and 3 women; average age, 58 a ± 7 a) with CHF (group B) were treated with digitalis and diuretics only. Additional 6 subjects (average age, 56 a ± 5 a), with no history of cardiac disease, hypertension or other cardiovascular disorders, had early

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morning resting blood specimens obtained as control. All patients and control subjects did not receive calcium blockers, angiotensin converting enzyme inhibitors (ACEI), β-AR blockers, and anti-platelet agents such as aspirin and dipyridamole in preceding 3 weeks. For all patients, platelet $[Ca^{2+}]_i$, lymphocyte β-AR density, and plasma NE concentration were measured before and after 14-d treatment.

Measurement of plasma norepinephrine Blood was collected from an indwelling venous catheter. Resting, fasting supine early morning samples were obtained for measuring NE levels by radioimmunoassay described by William and Ranm[13].

β-AR Radioligand binding assay Heparinized blood 20 mL was obtained and layered carefully on the top of the FicollPaque solution in a centrifuge tube. The two-phase system was centrifuged at 400 × *g* for 30 min. The mononuclear leukocytes (MNL) layer at the interface was collected and suspended in Hanks' Balanced Salt Solution (HBSS) and centrifuged at 250 × *g* for 10 min. The pellet of MNL was suspended in HBSS and centrifuged again. The final pellet was suspended in 3 mL HBSS and adjusted to 1×10^{10} cells/L. The resulting lymphocyte suspension 100 μL was incubated with 50 mL [^{3}H]dihydroalprenolol (DHA, final concentration 12 μmol/L) for 30 min at 4 ℃ in a Hanks-tris-HCl (50 mmol/L, pH 7.4) buffer. After rapid filtration through F_{49} filter paper and washing out twice with 5 mL incubation buffer, the filter paper was dried at 80 ℃ for 20 min. The radioactivity was counted using a Packard 4000 scintillator. Nonspecific binding was defined by co-incubation with propranolol 1.2 mmol/L. The density of β-AR receptors was calculated after normalization of the specific binding activity by lymphocyte counting.

Measurement of $[Ca^{2+}]_i$ in platelets Platelets were isolated by density gradient centrifugation and suspended in 3 mL HEPES solution (cantaining NaCl 134, KCl 2.9, $NaHCO_3$ 12, NaH_2PO_4 0.36, $MgCl_2$ 1, glucose 5, HEPES 5 mmol/L, and BSA 0.35 %, pH7.2). The solution was loaded with Fura 2-AM (1.25 mmol/L) at 37 ℃ for 30 min. The extracellular calcium was adjusted by adding $CaCl_2$ 1 mmol/L. The samples were centrifuged to collect the platelets. Then platelets were incubated in HEPES solution and adjusted to 1×10^8 cells/L. Fura 2-Ca^{2+} fluorescence (F) was measured with an F-3000 spectrophotometer with λ_{ex} 340 nm and λ_{em} 540 nm. The cells were treated with 0.1 % Triton-100 followed by the addition of $MnCl_2$ 1 mmol/L to obtain the maximal and minimal fluorescences, respectively. $[Ca^{2+}]_i$ was calculated according to the equation $F = K_d(F(F_{min})/(F_{max}(F)$, $K_d = 224$ nmol/L.

Reagents Diltiazem was purchased from Tianjin TianBian Company of China. Fura 2-AM and propranolol were purchased from Sigma Chemical Co. [^{3}H]-DHA (specific activity 1.55 pBq/mol) was obtained from China Institute of Atomic Energy, Beijing.

Statistical analysis Data were expressed as $\bar{x} \pm s$ and analyzed with one-way ANOVA (Graphpad Prism Software), and with a P value of 0.05 deemed statistically significant.

RESULTS

Plasma NE concentration and $[Ca^{2+}]_i$ in platelets were both higher in CHF patients than those in control, meanwhile, lymphocyte β-AR density was significantly lower in CHF patients than that in control (Tab 1). After treatment with diltiazem for 14 d, the β-AR density significantly increased ($P < 0.01$) and the platelet $[Ca^{2+}]_i$ levels markedly decreased ($P < 0.01$), although β-AR density was still lower, and $[Ca^{2+}]_i$ levels were

Tab 1. Changes of plasma NE levels, lymphocyte β-AR density and platelet $[Ca^{2+}]_i$ before and after treatment with diltiazem. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control. $^dP > 0.05$, $^fP < 0.01$ *vs* pre-treatment. Group A, treated with diltiazem. Group B, treated without diltiazem.

	Control ($n = 6$)	Group A ($n = 10$)		Group B ($n = 7$)	
		Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
NE (ng/L)	201 ± 10	251 ± 10^c	250 ± 9^{cd}	257 ± 11	250 ± 10^{cd}
β-AR (fmol/10^7 cells)	756 ± 131	440 ± 101^c	626 ± 35^{cf}	407 ± 74	410 ± 90^{cd}
$[Ca^{2+}]_i$(nmol/L)	94 ± 5	150 ± 9^c	126 ± 11^{cf}	149 ± 8	145 ± 8^{cd}
r	−0.36	−0.98	−0.73	−0.92	−0.94

still higher than that of control ($P < 0.01$, respectively). There were no significant changes in plasma NE levels after treatment with diltiazem. There was a negative correlation between lymphocyte β-AR density and platelet $[Ca^{2+}]_i$ levels ($r = -0.98$, $P < 0.01$ pre-treatment; $r = -0.73$, $P < 0.05$ post-treatment). There was no significant change in either β-AR density or platelet $[Ca^{2+}]_i$ levels ($P > 0.05$) in patients treated only with digitalis and diuretics.

DISCUSSION

The mechanism of β-AR down-regulation of cardiomyocytes and lymphocytes in patients and animal models with CHF is still not completely clear. Most of researches suggest that overactivated sympathetic nerve and increased plasma NE concentration are main causes[5]. Our recent study in cultured rat ventricular cardiomyocytes[12] showed that β-AR down-regulation induced by NE was related to an increased cardiomyocyte $[Ca^{2+}]_i$, and calcium channel antagonist verapamil could partly reverse this β-AR down-regulation induced by NE, meanwhile, also decrease intracellular $[Ca^{2+}]_i$. Moreover, $[Ca^{2+}]_i$ decrease was prior to β-AR increase. These results suggest that increased $[Ca^{2+}]_i$ in cardiomyocytes may be one of factors which causes β-AR down-regulation.

Since β-AR density of lymphocyte highly correlates with that of cardiomycytes in CHF and can be taken as a window for understanding the β-AR density changes in cardiomyocytes[14,15], and there are expressions of calcium channels on platelet membrane just like that in cardiomyocytes[16], in our present research, we attempt indirectly to understand the changes of β-AR and $[Ca^{2+}]_i$ of cardiomycytes through measuring β-AR density of lymphocytes and $[Ca^{2+}]_i$ in platelets. From the present study we found that in patients with CHF, calcium channel blocker diltiazem could increase lymphocyte β-AR density and decrease intracellular $[Ca^{2+}]_i$ in platelets, however, no significant change was found in plasma NE concentration after treatment. We also observed that there was a negative correlation presented between lymphocyte β-AR density and platelet $[Ca^{2+}]_i$. These results suggested that diltiazem could partly reverse the β-AR down-regulation of patients with CHF, and this effect of diltiazem might be attributed to the decrease of intracellular $[Ca^{2+}]_i$.

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地尔硫䓬对慢性充血性心力衰竭患者淋巴细胞膜β肾上腺素受体下调的影响[1]

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关键词 β肾上腺素受体；地尔硫䓬；钙；去甲肾上腺素；去甲肾上腺素；充血性心力衰竭

目的：研究钙拮抗剂地尔硫䓬是否逆转慢性充德性心力衰竭(CHF)患者淋巴细胞膜β肾上腺素受体(β-AR)的下调．**方法**：同位素放射配基法测定地尔硫䓬治疗前后 CHF 患者淋巴细胞 β-AR 密度，Fura 2-AM 荧光指示法测定血小板胞内$[Ca^{2+}]_i$，放射免疫法测定血浆去甲肾上腺素(NE)浓度的变化．**结果**：CHF 患者淋巴细胞 β-AR 密度低于对照组．血小板$[Ca^{2+}]_i$及血浆 NE 水平均高于对照组．地尔硫䓬降低血小板$[Ca^{2+}]_i$，升高淋巴细胞 β-AR 密度．治疗前后血浆 NE 水平无显著变化．无论治疗前后，血小板$[Ca^{2+}]_i$与淋巴细胞 β-AR 密度均呈负相关．**结论**：地尔硫䓬能够部分逆转心衰患者 β-AR 的下调，这种作用与血浆 NE 水平变化无关，而可能与降低细胞内$[Ca^{2+}]_i$有关．

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