Effects of BRL-37344 on expression of β-adrenoreceptors mRNA of rats with heart failure

KONG Yi-Hui*, LI Wei-Min, TIAN Ying, GAN Run-Tao, SUN Gui-Fang
(*Department of Cardiology, the First Clinical College, Harbin Medical University, Harbin* 150001, *China*)

Abstract: **AIM** To investigate the influence of β_3-adrenoreceptor(β_3-AR) and the effect of β_3-AR agonist on the failing hearts. **METHODS** Rats were randomly divided into control group, isoprenaline (Iso) group and Iso + BRL group. Iso group and Iso + BRL group received two sc injections of Iso (340 $mg \cdot kg^{-1}$) with a 24 h interval to induce heart failure. After 8 weeks, Iso + BRL group was given BRL-37344 0.4 $nmol \cdot kg^{-1} \cdot min^{-1}$ via tail-vein for 10 min, twice a week for 2 or 6 weeks. Mortality, hemodynamics, ratio of left ventricle weight and body weigh (LVW/BW) and levels of β_1-, β_2- and β_3-AR mRNA were measured at the tenth and the fourteenth week after injection of Iso. **RESULTS** There was no significant difference in the mortality among the three groups($P > 0.05$). Left ventricular end systolic pressure (LVESP), $\pm dp/dt_{max}$ decreased, and left ventricular end diastolic pressure (LVEDP), LVW/BW increased dramatically in Iso group. When compared with Iso hearts, Iso + BRL group had more deteriorated cardiac functions and higher LVW/BW. The level of β_1-AR mRNA was low and the level of β_3-AR mRNA was high in Iso group. When compared with Iso hearts, Iso + BRL group had lower level of β_1-AR mRNA and higher level of β_3-AR mRNA. The level of β_2-AR mRNA had no significant difference among three groups. **CONCLUSION** In failing hearts, reduction in β_1-AR mRNA and increase in β_3-AR mRNA might contribute to cardiac dysfunctions; the level of β_3-AR mRNA is higher than that in control heart; β_3-AR agonist aggravates cardiac dysfunctions.

Key words: receptors, adrenergic, β; RNA, messenger; heart failure; isoprenaline; BRL-37344

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Biographies: KONG Yi-Hui(1967 –), female, native of Anqing, Anhui, associate professor, Doctor of Medicine, main research field is cardiovascular pharmacology; LI Wei-Min(1949 –), male, professor, Postdoctor of Medicine, main research field is cardiovascular pharmacology.

* Corresponding author. E-mail: kongyihui@yahoo.com
Tel: (0451)53643849-5462

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Adrenergic receptors (AR) widely exist in the cardiovascular system. They have played important roles not only in physiological state, but also in the pathological course of myocardial hypertrophy, chronic heart failure (CHF), and atherosclerosis. AR have been classified into α-AR and β-AR. β-AR are the strongest stimulators in regulating cardiac functions[1]. Three distinct subtypes of β-AR, β_1-, β_2-, and β_3-AR, have been identified in the heart by classifying in pharmacologic means and molecular cloning[2]. Some studies about the effects of β_3-AR in heart failure have been reported[3,4]. This study concentrates on evaluating the effects of BRL-37344 [4-[-[2-hydroxy-(3-chlorophenyl) ethyl-amino] propyl] phenoxy- acetate], a β_3-AR agonist[3], on β_1-, β_2- and β_3-AR mRNA levels of rats with heart failure and investigating the influence of levels of β_1-, β_2-, and especially β_3-AR on failing heart.

1 MATERIALS AND METHODS

1.1 Drugs

BRL-37344 and isoprenaline (Iso) were gifts from Professor CHENG Che-Ping, the chairman of The Department of Cardiology of Bowman Gray School of Medicine, Wake Forest University, USA. One-step total RNA extraction kit was purchased from Gibco/BRL, Cergy Pontoise, France.

1.2 Heart failure model and drug administration

Male Wistar rats 10 – 12 weeks old, weigh-

ing 200 – 220 g, were purchased from the Experimental Animal Center of Harbin Medical University. One hundred and ten rats were randomly divided into two groups: control group (n = 20) and heart failure group (n = 90). The heart failure group received two subcutaneous injections of Iso (340 mg·kg^{-1}) with a 24 h interval. After 8 weeks, 40 survival rats in the heart failure group were divided into two subgroups: Iso group (n = 18) and Iso + BRL group (n = 22). Iso + BRL group was given BRL-37344 0.4 nmol·kg^{-1}·min^{-1} dissolved in sterile water from tailvein for 10 min, twice a week for 2 or 6 weeks. Rats in Iso group were injected with an equivalent volume of normal saline only at the same time. Control group received no administration.

1.3 Mortality rate

Mortality rate was assessed at the tenth or the fourteenth week after injection of Iso and BRL.

1.4 Hemodynamic measurements

Rats were anesthetized with 40 mg·kg^{-1} ketamine plus 10 mg·kg^{-1} xylazine, the right carotid artery was cannulated with a micro-tip pressure transducer (model SPC-320, Millar Instruments, USA). Under continuous pressure monitoring, the arterial catheter advanced retrogradely into the left ventricle (LV), left ventricular end systolic pressure (LVESP) and left ventricular end diastolic pressure (LVEDP) were recorded. The time constant of isovolumic LV relaxation (T_C) and maximal rates of rise and decline of ventricular pressure ($\pm dp/dt_{max}$) was calculated.

1.5 Left ventricle weight and body weight measurement

After hemodynamic measurement, the rats were sacrificed by injection of 2 mol·L^{-1} KCl (1 mL each rat). The body and LV were weighed. The ratio of LV weight and body weight (LVW/BW) was calculated.

1.6 Expression of β-AR mRNA detected by reverse transcriptase-polymerase chain reactions (RT-PCR)

The total RNA was extracted by one-step total RNA extraction kit. The conditions of RT-PCR were according to the method of Dincer, *et al*[5]. The PCR reaction condition of amplification and quantitation of mRNA encoding β_1-, β_2-, and β_3-AR in rat hearts are shown in Tab 1. β-actin was amplified in each set of PCR reactions, and these genes were served as internal references during quantitative analysis to correct operator and/or experimental variations. At the end of the reactions, 5 μL of each PCR sample was loaded onto a 2% agarose gel and electrophoresed for 2 h at 100 V. Afterwards, the resulting gels were scanned using an UV transilluminator (VDS, Pharmacia Biotech, Germany). Areas under the curves were measured and used as mRNA concentrations, and the ratios of β-AR mRNA to β-actin mRNA were calculated.

Tab 1. The polymerase chain reaction (PCR) reaction condition of amplification and quantitation of mRNA encoding β_1-, β_2-, and β_3-adrenoreceptor (AR) in rat hearts

Primer	Primer sequence 5′ – 3′	PCR product size/bp	Annealing temperature/℃
β_1-AR (sense)	GCCGATCTGGTCATGGGA		
β_1-AR (antisense)	GTTGTAGCAGCGGCGCG	327	58
β_2-AR (sense)	ACCTCCTCCTTGCCTATCCA		
β_2-AR (antisense)	TAGGTTTTCGAAGAAGACCG	560	60
β_3-AR (sense)	AGTGGGACTCCTCGTAATG		
β_3-AR (antisense)	CGCTTAGCTACGACGAAC	444	62
β-actin (sense)	CGTAAAGACCTCTATGCCAA		
β-actin (antisense)	AGCCATGCCAAATGTGTCAT	387	60

1.7 Statistical analysis

All data were presented as $\bar{x} \pm s$. One-way ANOVA and t test were applied in comparing differences.

2 RESULTS

2.1 Mortality

Eight weeks after two sc injections of Iso, rats were treated with BRL-37344 for 2 or 6 weeks. In the 10th week after Iso treatment, 1 rat in Iso group and 1 rat in Iso + BRL group were dead. In the 14th week, 2 rats in Iso group and 4 rats in Iso + BRL group were dead. But there was no significant difference among the mortalities in the three groups($P > 0.05$, Tab 2).

Tab 2. Mortality among three groups

Group	n	Mortality		
		9 – 10 week	11 – 14 week	Total
Control	20	0/10	0/10	0/20
Iso	18	1/9	2/9	3/18
Iso + BRL	22	1/11	4/11	5/22

Rats of isoprenaline (Iso) and Iso + BRL-37344 (BRL) groups received two sc injections of Iso (340 mg·kg^{-1}) with a 24 h interval to induce heart failure. Eight weeks later, Iso + BRL group was given BRL 0.4 nmol·kg^{-1}·min^{-1} dissolved in sterile water via tail vein for 10 min, twice a week for 2 or 6 weeks. Rats of Iso group were injected with an equivalent volume of normal saline only at the same time. Control group received no administration. Mortality was assessed at the 10th or the 14th week after injection of Iso. There was no significant difference among the three groups, $P > 0.05$.

2.2 Hemodynamic effects

Compared with the control group, dysfunction of heart was found in Iso group, as shown in Tab 3. LVESP, $\pm dp/dt_{max}$ were significantly lower, and T_C, LVEDP were significantly higher than those of control group. When compared with Iso group, dysfunction of heart was significantly aggravated in Iso + BRL groups, LVESP, $\pm dp/dt_{max}$ decreased, and T_C, LVEDP increased dramatically in Iso + BRL group.

2.3 Ratio of left ventricle weight and body weight

The LVW/BW in Iso and Iso + BRL groups was significantly higher than that in the control group at the 10th and the 14th week. When compared with Iso group, BRL-treated rats exhi- bited drastically greater heart weights and LVW/BW at the 14th week, as shown in Tab 4. There is no significant difference among body weight in three groups.

2.4 Expression of β_1-, β_2-, and β_3-AR mRNA

After electrophoresis with 2% agarose gels, the intensity of the 18S and 28S rRNA bands under UV light was routinely checked to verify that all samples were equally loaded and that no RNA degradation had occurred. Since the expression of β-actin mRNA among three groups remained the same, it proved that the effect of reverse transcriptase is consistent. Using β-actin genes as internal references, the ratios of β-AR mRNA and β-actin mRNA denoted the results of the expression of β_1-, β_2-, and β_3-AR mRNA(Fig 1).

Tab 3. Effect of BRL-37344 on hemodynamic parameters

Group	n	Time /week	HR /min^{-1}	LVESP /kPa	LVEDP /kPa	$+(dp/dt_{max})$ /kPa·s^{-1}	$-(dp/dt_{max})$ /kPa·s^{-1}	T_C /ms
Control	10	10	244 ± 14	15.0 ± 0.5	0.33 ± 0.03	1619 ± 127	− 1102 ± 94	12.9 ± 0.6
Iso	8	10	253 ± 16	13.1 ± 0.7**	1.23 ± 0.11**	1085 ± 113**	− 755 ± 70**	19.2 ± 1.2**
Iso + BRL	10	10	255 ± 13	12.4 ± 0.4**#	1.35 ± 0.04**##	963 ± 129**#	− 668 ± 884**#	20.2 ± 1.0**#
Control	10	14	242 ± 13	14.7 ± 0.5	0.35 ± 0.05	1600 ± 125	− 1088 ± 92	12.5 ± 0.6
Iso	7	14	248 ± 15	12.6 ± 0.7**	1.29 ± 0.07**	1070 ± 109**	− 721 ± 77**	19.8 ± 1.0**
Iso + BRL	7	14	257 ± 12	11.9 ± 0.4**#	1.37 ± 0.03**##	951 ± 92**#	− 653 ± 72**#	21.5 ± 0.9**#

See Tab 2 for rat treatments. Hemodynamic parameters were measured at the 10 th or the 14 th week after Iso and BRL injection. HR: heart rate; LVESP: left ventricular end systolic pressure; LVEDP: left ventricular end diastolic pressure; $\pm dp/dt_{max}$: maximal rates of rise and decline of ventricular pressure; T_C: time constant of LV relaxation. $\bar{x} \pm s$. ** $P < 0.01$, compared with corresponding control group; # $P < 0.05$, ## $P < 0.01$, compared with corresponding Iso group.

Tab 4. Effect of BRL-37344 on ratio of left ventricle weight (LVW) and body weight (BW)

Group	*n*	Time/ week	LVW/ mg	BW/ g	(LVW:BW)/ mg·g^{-1}
Control	10	10	532 ± 43	295 ± 20	1.80 ± 0.11
Iso	8	10	734 ± 53**	316 ± 36	2.33 ± 0.20**
Iso + BRL	10	10	702 ± 44**	306 ± 20	2.30 ± 0.17**
Control	10	14	639 ± 47	350 ± 33	1.82 ± 0.14
Iso	7	14	836 ± 57**	355 ± 34	2.37 ± 0.17**
Iso + BRL	7	14	896 ± 35$^{**\#}$	336 ± 25	2.68 ± 0.20$^{**}_{\#\#}$

See Tab 2 for rat treatments. $\bar{x} \pm s$. $^{**}P < 0.01$, compared with corresponding control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with corresponding Iso group.

The ratios of β_1-AR mRNA and β-actin mRNA were lowered, and the ratios of β_3-AR mRNA and β-actin mRNA were raised in Iso and Iso + BRL when compared with the control hearts (Tab 5). When compared with Iso hearts, Iso + BRL group has lower ratios of β_1-AR mRNA and β-actin mRNA and higher ratios of β_3-AR mRNA and β-actin mRNA at the 10th and the 14th week. The ratios of β_2-AR mRNA and β-actin mRNA also tended to be lower among three groups but with no significance.

3 DISCUSSION

The model of Iso-induced CHF has been widely used[6,7]. High doses of Iso (> 85 mg·kg^{-1}) produced a time-dependent and dose-

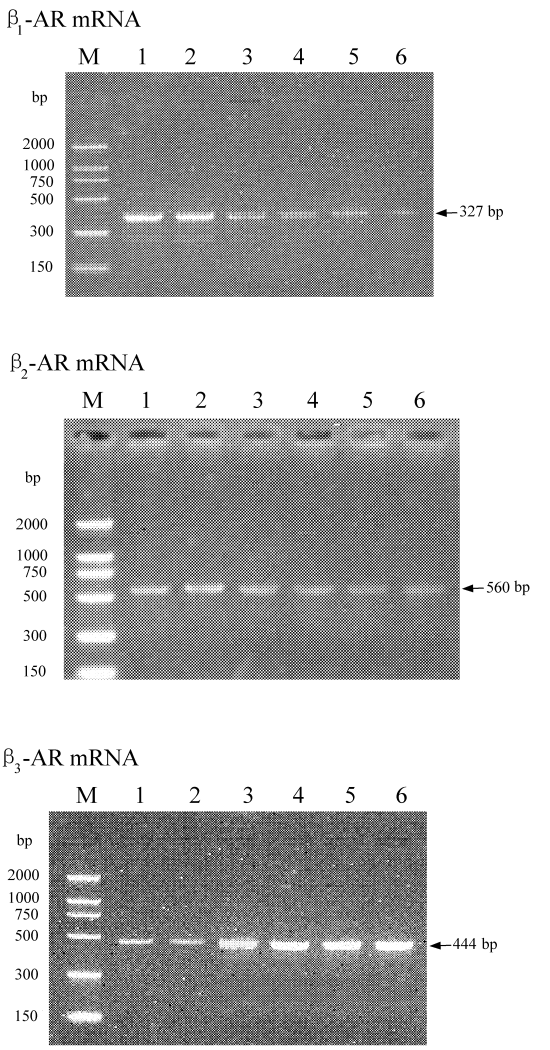

Fig 1. RT-PCR products obtained from rat hearts in control (lanes 1, 2), Iso (lanes 3, 4) and Iso + BRL (lanes 5, 6) group at the 10th week (lanes 1, 3, 5) and 14th week (lanes 2, 4, 6). M: marker.

Tab 5. Ratios of β-AR mRNA and β-actin mRNA

Group	Time /week	*n*	Ratio		
			β_1-AR:β-actin	β_2-AR:β-actin	β_3-AR:β-actin
Control	10	10	1.00 ± 0.13	0.33 ± 0.006	0.78 ± 0.10
Iso	10	8	0.68 ± 0.08**	0.31 ± 0.007	1.13 ± 0.15**
Iso + BRL	10	10	0.53 ± 0.13$^{**\#}$	0.30 ± 0.005	1.28 ± 0.08$^{**\#}$
Control	14	10	0.98 ± 0.12	0.32 ± 0.004	0.78 ± 0.13
Iso	14	7	0.59 ± 0.09**	0.30 ± 0.008	1.21 ± 0.10**
Iso + BRL	14	7	0.44 ± 0.08$^{**\#}$	0.29 ± 0.005	1.37 ± 0.09$^{**\#}$

See Tab 2 for rat treatments. $\bar{x} \pm s$. $^{**}P < 0.01$, compared with corresponding control group; $^{\#}P < 0.05$, compared with corresponding Iso group.

dependent impairment of cardiac functions including increase in right atrial pressure and left ventricular filling pressure, LV hypertrophy, and ventricular dilatation. Our study showed that Iso group had more deteriorated hemodynamics and higher LVW/BW when compared with control group on the fourteenth week after Iso injection. It further proved that a rat model of Iso-induced heart failure was accurate and reliable. No significant difference of mortality among the three groups may be due to the too small experimental rat samples.

When compared with Iso group, hemodynamics was worse, and LVW/BW was higher in BRL + Iso group. It further showed that the degree of CHF in BRL + Iso group was worse than that in Iso group. The negative inotropic effect of β_3-AR agonist exacerbates cardiac function. The studies regarding the effects of β_3-AR in CHF have been reported[3,4], but most of them were concentrated on acute medication in CHF. The study on chronic drug experiment has not been found as yet.

Low ratios of β_1-AR mRNA to β-actin and high ratios of β_3-AR mRNA to β-actin mRNA suggested that there exist the down-regulation of β_1-AR, no affection of β_2-AR and the upregulation of β_3-AR in CHF, which aggravated cardiac function. There are two reasons why β_1-AR down-regulated and β_2-AR unaffected in CHF. One is that the affinity of catecholamine and norepinephrine in CHF is 10 – 60 times higher for β_1-AR than for β_2-AR. Another is that β_1-AR is limited in synaptic cleft and may be in higher concentrations of norepinephrine. From these data, compared with β_1-AR, β_2-AR activation has little effect in CHF[1].

While compared with the ratio of Iso group, the ratio of Iso + BRL group was lower in β_1-AR mRNA and higher in β_3-AR mRNA. The negative inotropic effect of β_3-AR agonist aggravated cardiac dysfunction in CHF. In the normal heart, β_3-AR may exert a negative regulation against excessive positive inotropic stimulation, thereby moderating oxygen consumption, preventing calcium overload and ultimately cardiomyocyte toxicity. At early stages of cardiac dysfunction, endogenous NO production may, in addition to attenuating β_1- and β_2-adrenergic inotropic responses, improve diastolic relaxation[8], thereby compensating systolic dysfunction by increasing diastolic reserve. In terminally failing hearts, the residual negative inotropic effect may become maladaptive and aggravate systolic dysfunction. The treatment of BRL-37344 accelerates the increase in β_3-AR number, and the proportion of three β-AR may be changed. The density and product of β_1-AR decrease accordingly. It aggravates systolic and diastolic dysfunction deeply.

A definitive statement regarding the importance of the β_3-AR in the pathogenesis of CHF cannot be drawn. β_3-AR is refractory to short-term agonist-promoted uncoupling of the signaling pathway. The receptor is also resistant to long-term down-regulation. Furthermore, norepinephrine has a relatively high affinity for the β_3-AR. In addition, the level of G_i proteins, implicated in the β_3-AR signaling, is elevated in the failing myocardium. Increasing G_i proteins result in an increase NO production and cGMP, and then attenuated cardiac contractility. There may be several reasons for upregulation of β_3-AR in CHF. These data suggest that following prolonged activation by the sympathetic nervous system, the β_3-AR-mediated response be preserved whereas the β_1- and β_2-AR-mediated responses be diminished[9]. According to this hypothesis, the β_3-AR-mediated pathway would function as a countervailing "rescue" mechanism preventing myocyte damage from excessive stimulation of β_1- and β_2-AR. But as heart failure progresses to a later stage, this compensatory mechanism might become maladaptive, with a persistent negative inotropic effect, and lead to further myocardial depression.

The explanation of β_3-AR effect in the CHF are likely to shed new light on deeper understanding. The study of β_3-AR subtype enlightens the exploitation of drug and the new thought of thera-

py. It suggests that β-blockers specifically targeting β_1- and β_2-AR be more appropriate to preserve the countervailing β_3-AR-mediated pathway at earlier stages of the disease. Conversely, the β_3-AR-mediated pathway might become maladaptive, and nonspecific β-blockers and/or specific β_3-AR antagonists might be more desirable.

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BRL-37344 对心力衰竭大鼠 β 肾上腺素受体表达水平的影响

孔一慧, 李为民, 田　颖, 甘润韬, 孙桂芳

(哈尔滨医科大学第一临床医学院心内科, 黑龙江 哈尔滨　150001)

摘要: 目的　探讨 β_3-肾上腺素受体(β_3-AR)及其激动剂 BRL-37344(BRL)在心力衰竭中的作用。**方法** 大鼠分为对照组、异丙肾上腺素(Iso)组和 Iso + BRL 组。Iso 组和 Iso + BRL 组大鼠间隔 24 h, sc Iso 340 $mg\cdot kg^{-1}$ 2 次制作心力衰竭模型。8 周后, Iso + BRL 组大鼠从尾静脉给予 BRL 0.4 $nmol\cdot kg^{-1}\cdot min^{-1}$, 10 min, 每周 2 次, 给药 2 或 6 周。分别于给 Iso 后 10 或 14 周测定死亡率、血流动力学、左室重/体重、心肌组织 β_1-, β_2-和 β_3-AR mRNA 水平。**结果**　14 周时对照组、Iso 组和 Iso + BRL 组分别死亡 0/20, 3/18 和 5/22($P>0.05$)。Iso 使左室收缩末压、$\pm dp/dt_{max}$ 明显降低, 左室等容舒张时间常数、左室舒张末压明显增高, 左室重/体重明显增加; 与 Iso 组比, Iso + BRL 组的心脏舒缩功能进一步下降, 左室重/体重进一步增加。Iso 组 β_1-AR mRNA 水平降低, β_3-AR mRNA水平升高, 与 Iso 组比较, Iso + BRL组 β_1-AR mRNA 水平更低, β_3-AR mRNA水平更高。β_2-AR mRNA 3 组均有下降趋势, 但无统计学差异。**结论** 衰竭心脏β_1-AR 水平下降及 β_3-AR 水平增高可导致心功能降低。β_3-AR mRNA 水平在衰竭心脏比非衰竭心脏明显增高, 应用 β_3-AR 激动剂可明显加重心力衰竭。

关键词: 受体, 肾上腺素, β; RNA, 信使; 心力衰竭; 异丙肾上腺素; BRL-37344

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