# Effects of acute injection of BRL 37344 on hemodynamics and β-adrenoreceptors expression in myocardium of rats with heart failure

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**Abstract**: **AIM** To observe whether acute stimulation of BRL 37344, a $\beta_3$-adrenergic receptor ($\beta_3$-AR) agonist, has the same effects of exacerbating hemodynamics and increasing in $\beta_3$-AR expression on rats with failing heart as chronic administration. **METHODS** Rats received two doses of isoprenaline (Iso, 340 mg·kg$^{-1}$, sc, with a 24-h interval) to prepare heart failure model. After 8 weeks, rats were given iv BRL 37344 0.4 nmol·kg$^{-1}$·min$^{-1}$ for 10 min. Hemodynamics were measured at 0, 10, 30 min, 1, 2, 3 h, 1, 2 and 7 d after BRL 37344. Levels of β-AR mRNA in myocardium were measured at 0, 1, 2 and 7 d after BRL 37344 by reverse transcription-polymerase chain reaction. **RESULTS** Compared with Iso group, heart rate, left ventricular end systolic pressure and $+dp/dt_{max}$ were significantly higher, and left ventricular end diastolic pressure was significantly lower during 1-3 h after BRL 37344 injection in Iso + BRL group. Then, they were restored to the same level as that prior to BRL 37344 injection. Compared with normal control, the levels of $\beta_1$-, $\beta_2$- and $\beta_3$-AR mRNA displayed no significant change in BRL group; the level of $\beta_1$-AR mRNA was lower and the level of $\beta_3$-AR mRNA was higher in Iso group. In Iso + BRL group, much more lower $\beta_1$-AR mRNA level and much higher $\beta_3$-AR mRNA level were shown on d 2 and d 7 than Iso group. **CONCLUSION** Acute administration of $\beta_3$-AR agonist has a shorter improved hemodynamics. But it caused the same result as chronic administration in reduction of $\beta_1$-AR mRNA and increment of $\beta_3$-AR mRNA in failure hearts, which may aggravate the cardiac functions.

**Key words**: receptors, adrenergic, β; gene expression; heart failure; BRL 37344

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β-Adrenergic receptors (β-AR) are most important in regulating cardiac functions[1]. Some reports dealt with the effects of $\beta_3$-AR agonist exacerbating cardiac function in heart failure[2,3]. The negative inotropic effect of $\beta_3$-AR stimulation was mediated by activation of the nitric oxide synthase pathway. Some reports concluded that the levels of $\beta_3$-AR showed a remarkable increment in failing heart. The reduction of $\beta_1$-AR and the increment of $\beta_3$-AR may contribute to impairment of cardiac functions. The effects of $\beta_3$-AR were concentrated on chronic drug experiment in congestive heart failure[4], the effect of acute medication has not been reported. In this study, we used isoprenaline (Iso)-induced failing heart[5] to evaluate potential mechanisms of BRL 37344, a specific $\beta_3$-AR agonist[6] and determine the effects of $\beta_3$-AR on the failing heart.

## 1 MATERIALS AND METHODS

### 1.1 Drugs

BRL 37344 and Iso were provided by Professor CHENG Che-Ping, the chairman of Department of Cardiology of Bowman Gray School of Medicine, Wake Forest University, USA. One-

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step total RNA extraction kit was purchased from Gibco/BRL at Cergy Pontoise in France.

### 1.2 Animal

Male Wistar rats 10 – 12 weeks old, weighing 200 – 220 g, were purchased from the Experimental Animal Center of Harbin Medical University. The rats ($n$ = 174) were randomly divided into four groups: control group ($n$ = 28), BRL group ($n$ = 31), Iso group ($n$ = 55), and Iso + BRL group ($n$ = 60). Iso and Iso + BRL groups received two subcutaneous injections of Iso (340 mg·kg$^{-1}$) with a 24-h interval, as previously described[5]. After 8 weeks, BRL and Iso + BRL groups were given 1 μmol·L$^{-1}$ BRL 37344 0.4 nmol·kg$^{-1}$·min$^{-1}$ dissolved in sterile water from the tail vein for 10 min. At the same time, the rats in control and Iso groups were injected with only an equivalent volume of sterile water.

### 1.3 Hemodynamic measurements

The rats were anesthetized with 40 mg·kg$^{-1}$ketamine plus 10 mg·kg$^{-1}$ xylazine. Then a micro-tip pressure transducer (model SPC-320, Millar Instruments, USA) was inserted into the right carotid artery. Under continuous pressure monitoring, the arterial catheter advanced into the left ventricle. The heart rate, left ventricular end systolic pressure (LVESP), left ventricular end diastolic pressure (LVEDP), and maximal rate of rise of ventricular pressure ($+dp/dt_{max}$) were recorded at 0, 10, 30 min, 1, 2, 3 h, 1, 2, and 7 d after BRL or sterile water injections.

### 1.4 Expression of $\beta_1$-, $\beta_2$-, $\beta_3$-AR mRNA by reversing transcriptase-polymerase chain reactions (RT-PCR)

After hemodynamic measurements, the rats were decapitated. About 100 mg myocardium was stored into liquid nitrogen. The one-step total RNA extraction kit extracted the RNA at 0, 1, 2, and 7 d after BRL 37344 or sterile water injections. The conditions of RT-PCR were according to Dincer's method[7], as shown in Tab 1. β-Actin was amplified in each set of PCR, and these genes 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 was electrophoresed for 2 h at 100 V. Afterwards, the resulting gels were visualized using an UV transilluminator (VDS, Pharmacia Biotech, Germany). Areas under the curves were measured and were used as mRNA concentrations, and the ratios of β-AR mRNA to β-actin mRNA were calculated.

**Tab 1. Primers used in polymerase chain reaction (PCR) for amplification and quantitation of mRNA encoding $\beta_1$-, $\beta_2$-, and $\beta_3$- adrenergic receptors (AR) in rat hearts**

| Primer temperature/℃ | Primer sequence 5′ – 3′ | PCR product/bp | Annealing |
|---|---|---|---|
| $\beta_1$-AR (sense) | GCCGATCTGGTCATGGGA | | |
| $\beta_1$-AR (antisense) | GTTGTAGCAGCGGCGCG | 327 | 58 |
| $\beta_2$-AR (sense) | ACCTCCTCCTTGCCTATCCA | | |
| $\beta_2$-AR (antisense) | TAGGTTTTCGAAGAAGACCG | 560 | 60 |
| $\beta_3$-AR (sense) | AGTGGGACTCCTCGTAATG | | |
| $\beta_3$-AR (antisense) | CGCTTAGCTACGACGAAC | 444 | 62 |
| β-Actin (sense) | CGTAAAGACCTCTATGCCAA | | |
| β-Actin (antisense) | AGCCATGCCAAATGTGTCAT | 387 | 60 |

### 1.5 Statistical analysis

All data were presented as $\bar{x} \pm s$. One-way ANOVA and Turkey's test were applied in comparing differences among groups. Differences were considered to be significant at a value of $P < 0.05$.

## 2 RESULTS

### 2.1 Hemodynamic effects

Eight weeks after the Iso injections, 29 rats in Iso group and 30 rats in Iso + BRL group were left. After BRL 37344 treatment, 1 rat on d 2 and 4 rats on d 7 died in Iso + BRL group. There was no dead rat in other groups at different time after BRL 37344 injection.

As showed in Fig 1, there were no significant differences between the heart rate in Iso group and control group for most time. In Iso group, LVESP and $+dp/dt_{max}$ were significantly lower and LVEDP were significantly higher than that of control group. BRL 37344 infusion for 10 min induced a rapid increase in heart rate in BRL and Iso + BRL groups. It lasted for 1 h in BRL group. Increased heart rate in Iso + BRL group was greatly higher in comparison with Iso group, which lasted for 3 h after the injection. After 1 d, the heart rates restored to the same level as that prior to injection ($P > 0.05$). LVESP and $+dp/dt_{max}$ values in both BRL and Iso + BRL groups began to rise after BRL 37344 infusion. LVESP reached the maximum at 30 min and lasted for 2 h in BRL group and for 1 h in Iso + BRL group after injection. After 1 d, the LVESP restored to the level prior to injection. Increment of $+dp/dt_{max}$ lasted for 2 h in BRL group and for 1 h in Iso + BRL after injection, then restored to the level prior to injection.

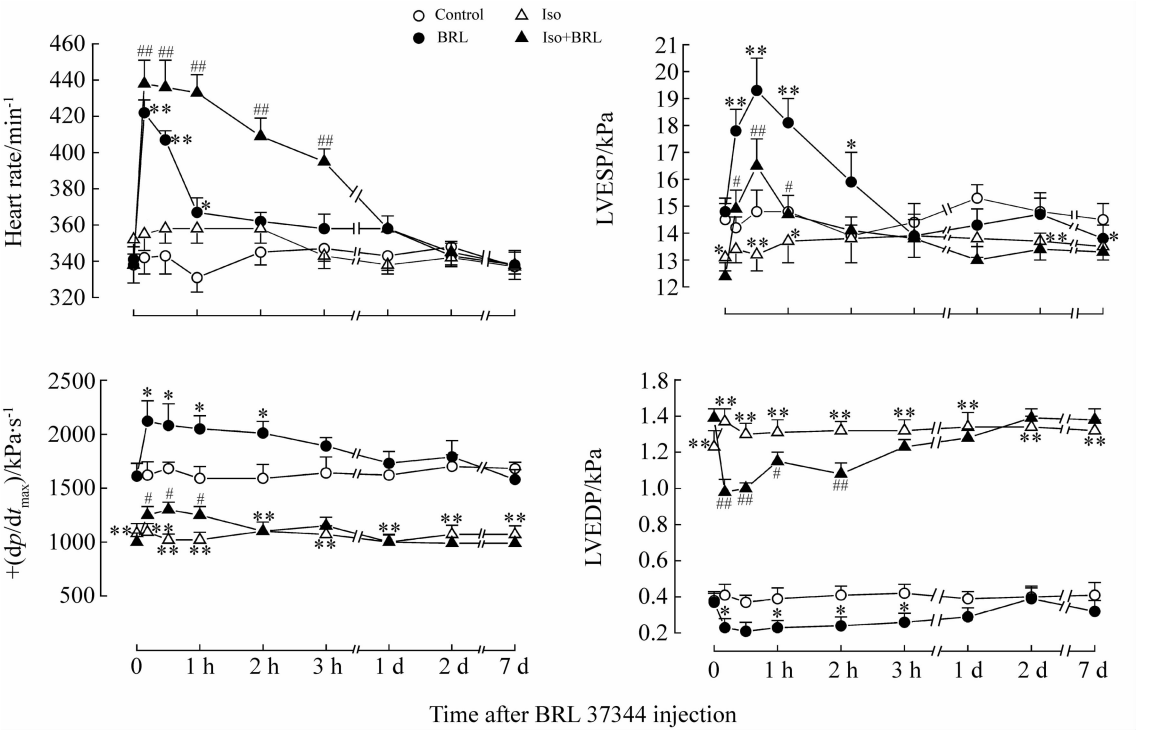


**Fig 1. Hemodynamics after acute injection of BRL 37344 in rats with heart failure.** Isoprenaline (Iso) and Iso + BRL groups received two subcutaneous injections of Iso (340 $mg \cdot kg^{-1}$) with a 24-h interval. After 8 weeks, the rats in BRL and Iso + BRL groups were given iv BRL 37344 0.4 $nmol \cdot kg^{-1} \cdot min^{-1}$ for 10 min, and the rats in control and Iso groups were injected with an equivalent volume of sterile water. LVESP: left ventricular end systolic pressure; $+dp/dt_{max}$: maximal rate of rise of ventricular pressure; LVEDP: left ventricular end diastolic pressure. $\bar{x} \pm s$, $n = 4 - 7$. $^{*}P < 0.05$, compared with control group; $^{\#}P < 0.05$, compared with Iso group.

LVEDP began to decrease after BRL 37344 infusion in both BRL and Iso + BRL groups and lasted for 3 h in BRL group and for 2 h in Iso + BRL group after injection. Then, LVEDP restored to the level prior to injection.

### 2.2 Expression of $\beta_1$-, $\beta_2$-, and $\beta_3$-AR mRNA

Using β-actin gene as internal reference, the ratio of β-AR mRNA and β-actin mRNA represents the expression of β-AR mRNA (Fig 2, Tab 2). When comparing Iso hearts with controlled hearts, the ratios of $\beta_1$-AR mRNA to β-actin mRNA were lower and the ratios of $\beta_3$-AR mRNA to β-actin mRNA were higher. The ratios of β-AR mRNA and β-actin mRNA had no change at early stages after BRL 37344 injection in BRL group. Lower ratios of $\beta_1$-AR mRNA to β-actin mRNA and higher ratios of $\beta_3$-AR mRNA to β-actin mRNA were seen on d 7 after injection when compared with that of control group. Iso + BRL group had remarkably both lower ratios of $\beta_1$-AR mRNA to β-actin mRNA and higher ratios of $\beta_3$-AR mRNA to β-actin mRNA on d 2 when compared with Iso or BRL group, which greatly increased on d 7. The ratios of $\beta_2$-AR mRNA to β-actin mRNA tended to be the same among 4 groups.

## 3 DISCUSSION

Our study showed that hemodynamics of Iso group was deteriorated when compared with control group after eight weeks of the Iso injection. It proved that the rat model of Iso-induced heart failure was accurate and reliable. After 10 min of BRL 37344 injection, heart rate increased dramatically in BRL and Iso + BRL groups. Compared with the BRL group, there were higher heart rate and were sustained for a longer time in Iso + BRL group. When compared with control and Iso groups, BRL and Iso + BRL groups had better hemodynamics at 10 min after BRL 37344 injection. It can be concluded that acute injection of $\beta_3$-AR agonist ameliorates hemodynamics.

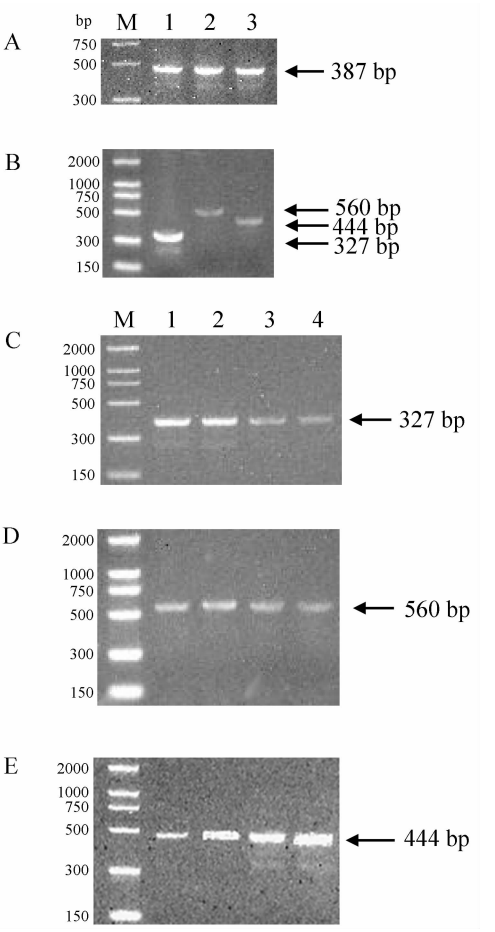


**Fig 2. Expression of β-AR mRNA in myocardium evaluated by RT-PCR.** A: Expression level of β-actin mRNA (lanes 1, 2, 3) at 0 min in control group; B: expression level of $\beta_1$-, $\beta_2$- and $\beta_3$-AR mRNA (lanes 1, 2, 3, respectively), at 0 min in control group; C, D and E: expression level of $\beta_1$-, $\beta_2$- and $\beta_3$-AR mRNA, respectively, at 7 d in control (lane 1), BRL (lane 2), Iso (lane 3) and Iso + BRL (lane 4) groups. M: marker.

The ratios of $\beta_1$-AR mRNA to β-actin mRNA were lower and the ratios of $\beta_3$-AR mRNA to β-actin mRNA were higher in Iso treated rat hearts in comparison with the control hearts. $\beta_2$-AR mRNA levels were apparently unaffected by heart failure. It can be concluded that not only the levels of $\beta_1$-AR have changed, but also the levels of $\beta_3$-AR show a remarkable increase in heart failure. This is consistent with previous studies about chronic effects[8]. Although $\beta_3$-AR agonist changed the gene expression, it occurred later than hemodynamic effects. Both lower ratios of $\beta_1$-AR mRNA to

**Tab 2.　Ratios of β-AR mRNA and β-actin mRNA**

| Group | Time/d | *n* | $\beta_1$-AR mRNA: β-actin mRNA | $\beta_2$-AR mRNA: β-actin mRNA | $\beta_3$-AR mRNA: β-actin mRNA |
|---|---|---|---|---|---|
| Control | 0 | 5 | 1.00 ± 0.13 | 0.33 ± 0.006 | 0.78 ± 0.10 |
| | 1 | 5 | 0.98 ± 0.14 | 0.33 ± 0.009 | 0.75 ± 0.14 |
| | 2 | 7 | 0.97 ± 0.12 | 0.32 ± 0.012 | 0.71 ± 0.11 |
| | 7 | 7 | 1.10 ± 0.15 | 0.35 ± 0.010 | 0.81 ± 0.12 |
| BRL | 0 | 7 | 1.03 ± 0.13 | 0.31 ± 0.007 | 0.88 ± 0.08 |
| | 1 | 5 | 1.00 ± 0.15 | 0.32 ± 0.006 | 0.90 ± 0.10 |
| | 2 | 7 | 0.98 ± 0.11 | 0.32 ± 0.009 | 0.91 ± 0.11 |
| | 7 | 7 | 0.87 ± 0.09 * | 0.34 ± 0.009 | 1.06 ± 0.12 * |
| Iso | 0 | 5 | 0.68 ± 0.08 * | 0.31 ± 0.007 | 1.13 ± 0.15 * |
| | 1 | 6 | 0.68 ± 0.09 * | 0.33 ± 0.010 | 1.16 ± 0.19 * |
| | 2 | 7 | 0.70 ± 0.10 * | 0.34 ± 0.008 | 1.16 ± 0.18 * |
| | 7 | 7 | 0.71 ± 0.11 * | 0.32 ± 0.011 | 1.21 ± 0.17 * |
| Iso + BRL | 0 | 5 | 0.63 ± 0.13 △ | 0.30 ± 0.005 | 1.18 ± 0.08 △ |
| | 1 | 5 | 0.59 ± 0.12 △ | 0.31 ± 0.009 | 1.20 ± 0.10 △ |
| | 2 | 6 | 0.31 ± 0.09 #△ | 0.31 ± 0.010 | 1.57 ± 0.12 #△ |
| | 7 | 5 | 0.24 ± 0.07 #△ | 0.32 ± 0.008 | 1.97 ± 0.19 #△ |

See legend of Fig 1 for rat treatments. $\bar{x} \pm s$. * $P < 0.05$, compared with control group; # $P < 0.05$, compared with Iso group; △ $P < 0.05$, compared with BRL group.

β-actin mRNA and higher ratios of $\beta_3$-AR mRNA to β-actin mRNA were seen on d 7 in BRL group and on d 2 and d 7 in Iso + BRL group after BRL 37344 injection. The level of β-AR mRNA changed earlier in failure hearts than in normal hearts. The reason may be that myocardial energy expenditure leads to tachycardia, myocardial hypertrophy, myocardial ischemia, and an overload of $Ca^{2+}$ in myocardium during a heart failure. The unbalance will emerge earlier after a large $\beta_3$-AR agonist infusion in reduction of $\beta_1$-AR mRNA and in augmentation of $\beta_3$-AR mRNA of failure heart. Increment of $\beta_3$-AR expression was seen in hearts with hemodynamic exacerbation in chronic experiment. If being stimulated repeatedly with $\beta_3$-AR agonist at this condition, the change in cardiac function and $\beta_3$-AR expression will be more obvious[4]. In acute experiment, an improved hemodynamic response to $\beta_3$-AR agonist was found at early stage, which is different from decreased heart function by the long-term effect. This indicates that although acute administration of $\beta_3$-AR agonist has a shorter improved hemodynamics, but the caused lower $\beta_1$-AR expression and higher $\beta_3$-AR expression result in a long exacerbated cardiac function in failure hearts. Further experiments are needed to prove the mechanism undergoing.

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# BRL 37344 急性用药对心力衰竭大鼠血流动力学和 β 肾上腺素受体表达的影响

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**摘要: 目的**　观察心力衰竭大鼠急性给予 $\beta_3$ 肾上腺素受体($\beta_3$-AR)激动剂 BRL 37344 是否与慢性给药一样,使 $\beta_3$-AR 表达进一步增加,心脏功能进一步恶化。**方法**　大鼠 sc 异丙肾上腺素 (Iso, 340 mg·kg$^{-1}$,2 次,间隔 24 h )制备心衰模型 。8 周后静脉给予 BRL 37344 0.4 nmol·kg$^{-1}$·min$^{-1}$, 10 min,测定给药后 0,10,30 min, 1, 2, 3 h, 1, 2 和 7 d 的血流动力学变化;逆转录聚合酶链反应方法测定 0,1, 2 和 7 d 的心肌组织 β-AR mRNA 水平。**结果**　与 Iso 模型组相比,Iso + BRL 组注射 BRL 37344 后 1 ~ 3 h 心率、+ $dp/dt_{max}$ 和左室收缩末压明显增加,左室舒张末压明显降低,之后恢复至注射 BRL 37344 前水平。与正常对照组相比,BRL 组注射 BRL 37344 后 $\beta_1$-,$\beta_2$-和 $\beta_3$-AR 水平变化不明显,Iso 组 $\beta_1$-AR mRNA 水平明显降低,$\beta_3$-AR mRNA 水平明显上升。Iso + BRL 组注射 BRL 37344 后 d 2 起,$\beta_1$-AR mRNA 水平较 Iso 组进一步降低,$\beta_3$-AR mRNA进一步上升,d 7 时变化更明显。**结论**　BRL 37344 急性用药对衰竭心脏血流动力学有短暂的改善作用,但与慢性给药同样使衰竭心脏 $\beta_1$-AR mRNA水平下降和 $\beta_3$-AR mRNA 水平上升,这有可能导致心脏功能的恶化。

**关键词:** 受体,肾上腺素,β; 基因表达; 心力衰竭; BRL 37344

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