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Fenoterol Enantiomers do not Possess Beneficial Therapeutic Properties of Their Racemic Mixture in the Rat Model of Post Myocardial Infarction Dilated Cardiomyopathy

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Abstract

Purpose—A salutary effect of β_2 adrenergic receptor (AR) agonist, fenoterol has been demonstrated in a rat model of post-myocardial infarction (MI) dilated cardiomyopathy (DCM). Recent reports on single cardiomyocyte experiments suggested that out of two enantiomers, RR and SS, that constitute a racemic mixture of fenoterol, only RR-enantiomer is an active component that might be a promising new drug for treatment of chronic heart failure. The objective of this study was to compare the efficacy of the RR enantiomer of fenoterol with efficacy of racemic fenoterol, and SS, an inactive enantiomer, in whole animal experimental models of DCM.

Methods—Two weeks after induction of MI by permanent ligation of the anterior descending coronary artery early cardiac remodeling and MI size were assessed via echocardiography and rats were divided into treatment groups. Treatment (placebo, racemic fenoterol, RR- or SS-enantiomers of fenoterol) continued for 6 months while progression of DCM was followed by serial echocardiography.

Results—Compared with untreated rats, rats treated with racemic fenoterol demonstrated previously described attenuation of LV remodeling, functional decline and the arrest of the MI expansion during the first two months of treatment. On the contrary, the treatment with either RR-, or with SS-enantiomers of fenoterol was completely ineffective.

Conclusion—The conclusion drawn on the basis of previous experiments with single cardiomyocytes that RR-enantiomer of fenoterol represents an active component of racemic fenoterol and can be further investigated as a new drug for treatment of chronic heart failure was not confirmed in the whole animal model of DCM.

Keywords

Chronic heart failure; cardiac remodeling; β_2 adrenergic receptor agonists; fenoterol; stereoisomers of fenoterol

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Introduction

Chronic heart failure (CHF) continues to be a major cause of mortality and represents a major reason for declining a quality of life, especially in older population [1]. Thus, the search of new or complimentary therapeutic strategies to reduce mortality and the progression of disease in CHF patients remains on the forefront of cardiovascular research. Results of multiple preclinical studies demonstrated a salutary effect of β_2 adrenergic receptor (AR) agonist, fenoterol as a complimentary therapy to a β_1 AR blocker [2, 3] or to a standard therapy for a CHF – combination of β_1 AR blocker and ACE inhibitor [4] in a rat model of post-myocardial infarction (MI) dilated cardiomyopathy (DCM). After induction of MI, one-year mortality in rats treated with a combination of β_1 AR blocker, metoprolol, and β_2 AR agonist, fenoterol, was significantly improved compared to mortality of untreated rats or those subjected to a monotherapy with either one drug, and was at least as good as mortality followed to a currently recommended therapy for CHF patients (β_1 AR blocker + ACE inhibitor). Cardiac remodeling and MI expansion following MI induction was significantly more attenuated in metoprolol+fenoterol treated rats than in rats under other tested treatments, and less apoptosis and higher β_1 AR density were observed when treatment was supplemented with fenoterol vs untreated animals [for review see 5]. A potential mechanistic basis for these preclinical trials was assumed from experiments on single cardiomyocytes indicating that activation of β_1 AR promotes apoptosis, while β_2 AR stimulation is cardioprotective [6–9].

Fenoterol, a β_2 AR agonist approved for treatment of bronchial asthma in Europe and Canada and marketed there under brand name Berotec (Boehringer-Ingelheim), exists as four stereoisomers. The combination of two enantiomers, RR and SS constitute a racemic mixture, which represents an actual drug that had been used clinically for treatment of asthma and for the aforementioned preclinical trials [2–4]. In order to refine the therapeutic effectiveness of fenoterol in the CHF model, its stereoisomers have been synthesized and tested with respect to their binding ability to β_2 AR, selectivity, and functional activity [10]. Experiments in isolated rat cardiomyocytes show that the RR enantiomer of fenoterol exhibits more potent effects on cardiomyocyte contraction and better Gs protein selectivity than other isomers [11, 12], suggesting that β_2 AR activity resides primarily with RR enantiomer of racemic fenoterol and that RR enantiomer might be a promising new drug for CHF [13]. The objective of this study was to test the efficacy of the RR enantiomer of fenoterol (RF) and compare it with efficacy of racemic fenoterol (F), for which the efficacy has been demonstrated in previous experiments, and SS, the least Gs selective enantiomer (SF), in whole animal experimental models of DCM.

Materials and Methods

Experimental design and procedures were in conformance with the NIH Guide for the Care and Use of Laboratory Animals, Manual 3040–2 (1999) and approved by Institutional Animal Care and Use Committee. It has been previously reported that the effect of racemic mixture of fenoterol to enhance the shortening of a single cardiomyocyte was entirely replicated by its RR-enantiomer [12]. Thus the effect of racemic fenoterol should be achieved with a half of the dose if its RR-enantiomer is used. To test this conclusion the

pilot acute experiment was conducted in 20 2-mo old male Wistar rats, weighing 350 g to 380 g (Charles River Inc., Wilmington, MA). Animals were anesthetized with 2% of Isoflurene. 1F pressure catheter (Millar instruments, Houston, TX) was inserted into abdominal aorta via femoral artery and systolic blood pressure was recorded on the PowerLab (ADInstruments, Colorado Springs, CO). A femoral vein was catheterized for drug infusion. The drugs dissolved in normal saline were infused intravenously for 5 minutes with a rate of 200 μ L/min. The concentration of racemic fenoterol resulting in ~10% reduction of systolic arterial blood pressure was established in the preliminary experiment (4 rats). Other 16 rats were divided into 4 groups, each being infused either with normal saline, or with racemic fenoterol (F) in the concentration established during preliminary testing, or with RR- or SS- enantiomers of fenoterol (RF, SF) in the half of concentration used for racemic fenoterol.

The main experiment was similar to previously described studies [2–4], except for the length of therapy. Briefly, the MI was induced surgically by a permanent ligation of the left descending coronary artery [2] in 100 2-mo old male Wistar rats, weighing 350 g to 380 g (Charles River Inc., Wilmington, MA). Additional 10 rats were subjected to a sham operation. Two weeks following surgery survivors were evaluated by echocardiography (Echo) under light Isoflurane anesthesia (1% in oxygen)[2]. At this time the early remodeling, functional loss, and MI size were assessed. Rats with MI size below 20% and exceeding 50% of LV were removed from the study. The MI rats were divided into four groups of similar average MI size and variability: non-treated (MI-nT); treated with a selective β_2 AR agonist (racemic fenoterol, MI-F); treated with RR-enantiomer of fenoterol (MI-RF); or treated with SS-enantiomer of fenoterol (MI-SF). Echo indices of LV volumes and function, as well as MI size at this time were considered as the pre-treatment baselines for each group.

Following the baseline Echo, i.e., 2 weeks after surgery, treatment was started. Drugs were dissolved in the drinking water. Water consumption was measured so that concentration of drugs could be adjusted in such a way that the daily dose was maintained at 250 μ g/kg for MI-F and 125 μ g/kg for MI-RF and MI-SF, respectively. Treatment continued for 6 months. Echo was repeated after 1, 2, 4, and 6 months of treatment. Sham-operated rats were echo-tested 2 weeks after surgery (before initiation of treatment) and at the end of experiments. Following the final, 6-mo Echo, all rats underwent an invasive hemodynamic study [14], and hearts were harvested for histological evaluation. Noninvasive tail blood pressure measurement (Kent Scientific, MA) was conducted under light Isoflurane anesthesia (1% in oxygen) at baseline in MI-nT group and after 1, 2, 4, and 6 months of treatment in all animals.

In additional experiment 60 2-mo old male Wistar rats were subjected to a coronary ligation and 2 weeks later survivals were divided into experimental groups as described above for the main experiment. The effect of treatment was tested in the group which received twice as much of RR-enantiomer of fenoterol that was used in the main experiment (MI-RF2, 250 μ g/kg) and in the group that was treated with a mixture of RR- and SS-enantiomer of fenoterol (MI-RFSF, 125 μ g/kg of each), and compared with untreated group (MI-nT). Baseline Echo was repeated after 1 and 2 months of treatment, at which time the experiment

was terminated. The length of additional experiment was based on the results of previous experiment [3] that demonstrated that salutary effects of racemic fenoterol on post-MI cardiac remodeling waned after 2 months of treatment. Fenoterol was purchased from Sigma, RR- and SS-enantiomers of fenoterol [13] were provided by Dr. Irving Wainer (Bioanalytical Chemistry and Drug Discovery Section, NIA).

Echocardiography

Echocardiography (Sonos 5500, a 12-MHz transducer; Hewlett Packard, Andover, MA) was conducted under light Isoflurane anesthesia (1.5% in oxygen) as described previously [2]. In brief, parasternal long axis views were obtained and recorded to ensure that the mitral and aortic valves and the apex were visualized. Short axis views were recorded at the midpapillary muscle level. Endocardial area tracings, using the leading edge method, were performed in a 2D-dimensional mode (short and long axis views) from digital images captured on cineloop to calculate end diastolic and end systolic LV areas. End-diastolic volume (EDV) and end-systolic volume (ESV) were calculated by a modified Simpson's method. EF was then derived as $EF = (EDV - ESV)/EDV \times 100$. The MI size at the midpapillary muscles level was estimated from 2D short axis LV images and expressed as a percentage of the LV endocardial circumference. Infarct area was identified as a sharply demarcated section of the LV free wall that failed to thicken during systole. The length of the akinetic part of the LV endocardial circumference was measured from freeze-frame images at end-diastole. Posterior wall thickness was measured from M-mode. All measurements were made by a single observer who was blinded to the identity of the tracings. All measurements were off-line averaged over three to five consecutive cardiac cycles. The reproducibility of measurements was assessed in two sets of baseline measurements in 10 randomly selected rats, and the repeated measure variability did not exceed $\pm 5\%$.

Hemodynamic Measurements

Invasive LV pressure-volume loop analyses were conducted as described previously (Ahmet et al., 2004). Rats were anesthetized with isoflurane (2% in oxygen), intubated, and ventilated. A bilateral thoracotomy was performed in the sixth intercostal space. A 1.4 French-combined pressure-conductance catheter (Millar Instruments Inc., Houston, TX) was inserted into LV through the apex. Traditional load-dependent hemodynamic indices, such as EF, $+dP/dt$, $-dP/dt$, end-diastolic pressure, and isovolumic relaxation time constant (τ), were measured, and load-independent indices, i.e., end-systolic elastance (Ees), preload recruitable stroke work (PRSW), and end-diastolic stiffness (Eed) were determined or calculated. Arterial elastance (Ea) was calculated as index of vascular tension. Arterioventricular coupling, an index of cardiac work efficiency, was calculated as Ea/Ees .

Histological Acquisition

Histological staining and analyses were performed as described previously [2]. In brief, the hearts were isolated and weighed. Myocardial segments from the midpapillary muscle level were imbedded in the paraffin, sectioned (5 μ m), and stained with Masson's trichrome and hematoxylin and eosin staining. MI size was expressed as an average percentage of the LV

endocardial and epicardial circumferences that were identified as infarct in the Masson's trichrome-staining sections.

Statistical Analysis

All data are expressed as the mean $\pm$ SEM. Echo-derived indices were compared via two-way analyses of variance (ANOVA) for repeated measures. Group differences at specific time points were assessed by a Bonferroni test. Differences in hemodynamic or histological data among MI-nT, MI-F and two stereoisomers, MI-RR and MI-SS were assessed by one-way ANOVAs, followed by a Bonferroni test. A $p < 0.05$ was considered statistically significant.

Results

Acute experiments

It was established in preliminary testing that ~10% reduction of systolic blood pressure is achieved by intravenous infusion of racemic fenoterol at the rate of 0.1 $\mu\text{g/kg/min}$. Results of an acute experiment comparing the effectiveness of racemic fenoterol and its enantiomers to reduce blood pressure are presented in Figure 1. Five minutes of intravenous infusion of normal saline at the rate of 200 $\mu\text{L/min}$ did not affect the systolic blood pressure. Racemic fenoterol (F) was infused for five minutes at the rate of 0.1 $\mu\text{g/kg/min}$, while its enantiomers (R or S) were infused at half of that dose, 0.05 $\mu\text{g/kg/min}$. After 1 min of infusion blood pressure was slightly reduced in all drug groups, but reduction was not statistically significant. However, after second minute of infusion statistically significant ($p < 0.01$) reduction of blood pressure was observed in F and R groups only. There was no difference in the extent of pressure reduction between F and R groups. Blood pressure in S group remained statistically not different from control, saline injected group.

Chronic experiments

Early mortality, cardiac remodeling, and treatment group assignment—

Perioperative mortality was 28% among coronary ligated animals (28 out of 100) and zero among sham operated rats. No animals were lost during the first 2 weeks after surgery. MI size was measured by Echo two weeks after coronary ligation. Fourteen rats whose MI size exceeded 50% or was less than 20% of LV were removed from the study. The remaining 58 rats were assigned to treatment groups, assuring equal average MI size and variability among groups as following: MI-nT, $n=12$; MI-F, $n=12$; MI-RF, $n=11$, and MI-SF, $n=11$.

Table 1 presents the results of Echo evaluation two weeks after MI induction, prior to initiation of treatment in coronary ligated and sham-operated animals, i.e., early remodeling and pretreatment baseline data. Table 1 demonstrates even distribution of animals among experimental groups with respect to average MI size and early LV remodeling. In comparison with Sh, End-diastolic (EDV) and end-systolic (ESV) LV volumes expanded in MI rats by 48-59% and 211-232% respectively, while ejection fraction fell by 51-55%. LV volumes and EF at this time were not different among MI groups, but significantly different from Sh. MI size was in the range of 29.6-31.7% of LV and also was not different among groups.

Mortality during 6 months of treatment—Total mortality during 6 months of observation was 13% and did not differ among experimental and untreated groups.

LV remodeling and MI expansion during 6 months of observation in untreated group—Results of serial Echo are presented in Figure 2. Compared to pretreatment baselines, the average EDV and ESV in nT group increased during the next 6 months by 60 and 79% respectively, while EF fell by 45%, MI size expanded by 42% during this time period. All changes were significantly different from baseline levels.

LV remodeling and MI expansion during 6 months of observation in treatment groups—In animals treated with racemic fenoterol (MI-F) LV volumes (Fig. 2) also increased compared with pretreatment baseline levels, by 55 and 68% for EDV and ESV respectively ($p<0.01$), EF fell by 19% ($p<0.05$), but MI size expanded by only 15% ($p>0.05$). However, the pattern of change during 6 months of treatment was remarkably different from MI-nT group. During the first 2 months of treatment, the EDV and ESV in MI-F group were significantly lower, and EF was significantly higher than in M-nT group ($p<0.05$). The MI size in MI-F group did not expand during the first 3 months of treatment and remained statistically below MI-nT level. These salutary effects observed during the first 2 months in MI-F group weakened after the second month of treatment and differences between MI-F and untreated groups disappeared at the end of experimental period.

In contrast to the M-F group, all Echo indices, including MI size for MI-RF and MI-SF groups were very similar to MI-nT group at every time point (Fig. 2).

MI size estimated from the last Echo and histologically at the conclusion of the study is presented on Fig. 3A. The coefficient of determination of histologically measured values of MI size on the basis of Echo-derived measurements was statistically significant ($R^2=0.7$). Indeed, the MI sizes measured histologically after completion of the experiment (Fig. 3B) were very similar to those presented in Fig. 2. While the MI size at 6 months was smallest in MI-F group, but differences among groups were not statistically significant.

Additional parameters during 6 months of observation

Since the experiment was conducted during the rats' period of rapid growth, the body weight increased during the 6 months of observation by approximately 78%. There were no differences in the rate of weight gain between untreated and different treatment groups. There were also no differences among groups in the heart rates (Echo) and blood pressure (tail cuff) during serial monthly measurements (Fig. 4) or pressure-volume (PV) loop analyses (Table 2) conducted at the end of observation. PV loop analyses revealed significant reduction of systolic and diastolic functions in untreated MI animals as compared with Sh, however, there were no improvement among treatment groups, with the exception of normalization of arterio-ventricular coupling (Ea/Ees) in MI-F group.

Additional control experiment

Perioperative and early mortality as well as early remodeling in rats subjected to a coronary ligation in additional experiment were similar to those in the main experiment. Two weeks

after surgery and after baseline Echo the assignment of survived rats to a treatment group was as following: MI-nT – 11, MI-RF2 – 12, MI-RFSF – 11. Results of Echo assessment of cardiac remodeling and MI expansion are presented in Fig. 5. Increase of LV volumes, decline of EF and expansion of MI size were similar in MI-nT and MI-RF2 groups. However, in the group treated with a mixture of enantiomers (MI-RFSF) the cardiac remodeling was significantly attenuated and LV volumes at the second month Echo were significantly lower than in MI-nT group ($p<0.05$). Moreover, EF in MI-RFSF group did not decline below baseline level and was higher than in MI-nT group ($p<0.05$). MI size in MI-RFSF group at the second month Echo was also less than in MI-nT group ($p<0.05$).

Discussion

Results of preliminary, acute experiments confirmed the previous conclusion that R-enantiomer of fenoterol entirely captures some properties of its racemic mixture and justified the 50% reduction doses for enantiomers in comparison with their racemic mixture in the main chronic experiment.

The results of the main chronic experiment, in great detail, replicated the previously reported therapeutic effects of racemic fenoterol in the model of post MI DCM in rats [2–5]. Specifically, treatment with fenoterol started 2 weeks following induction of MI attenuated LV remodeling and functional decline and arrested the MI expansion. Moreover, as had been reported previously, the therapeutic effects of chronic fenoterol treatment lasted for the first two month of treatment only, and then waned, when treatment continued beyond this time [3].

On the contrary, in the same experimental model, the treatment with either RR-enantiomer of fenoterol, which according to experiments on single cardiomyocytes is the only active isomer of fenoterol with respect to receptor binding and cardiomyocyte contraction, and which was the only enantiomer effectively reducing blood pressure in our acute experiment, or with SS-enantiomer, which is fully inactive [10, 11], was completely ineffective. Moreover, as was demonstrated in additional experiment (Fig. 5), the doubling of the dose of RR-enantiomer does not improve its effectiveness, while the treatment with a mixture of both enantiomers was as effective as a racemic fenoterol. While the ineffectiveness of SS-enantiomer in our study was fully anticipated, and in fact it was intended as a negative control, the lack of effectiveness on the part of RR-enantiomer contradicts the hypothesis promoted in the reports based on recording of its effect on contraction of single cardiomyocytes [12].

Mechanistic studies during the last decade provided numerous evidences that activation of β_2 AR is antiapoptotic and cardioprotective [6–9] and that effect might be related to its double coupling to Gs and Gi proteins [15–17]. Fenoterol contains two chiral centers and may exist in four stereoisomers – two enantiomers (RR and SS) and two RS isomers. In a recent study [12] these isomers were tested with respect to their selectivity to activate β_2 AR coupling to specific G proteins. It was shown that the RR enantiomer predominantly activates Gs coupling and thus results in maximum contraction of cardiomyocytes with and

without PTX. In contrast, the SR configuration is sensitive to PTX and thus activates coupling with both Gs and Gi.

The inability of the RR-enantiomer of fenoterol to attenuate the cardiac remodeling and functional deterioration in the rat model of post MI DCM indicates that coupling of β_2 AR to Gs is not sufficient for rescue myocardium in heart failure. The present results suggest that either, contrary to previously proposed theory [17], racemic fenoterol is not coupled predominantly to Gs, and partial Gi coupling plays role in its cardioprotective properties, or that mechanisms of cardioprotection by fenoterol are not Gi related and need to be studied further.

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References

1. Xu J, Kochanek KD, Murphy SL, Tejada-Vera B. Deaths: Final Data for 2007. NVSR. 2010; 58:1–135.
2. Ahmet I, Lakatta EG, Talan MI. Pharmacological stimulation of beta2-adrenergic receptors (beta2AR) enhances therapeutic effectiveness of beta1AR blockade in rodent dilated ischemic cardiomyopathy. *Heart Fail Rev.* 2005; 10:289–296. [PubMed: 16583177]
3. Ahmet I, Krawczyk M, Zhu W, Woo AY, Morrell C, Poosala S, et al. Cardioprotective and survival benefits of long-term combined therapy with beta2 adrenoreceptor (AR) agonist and beta1 AR blocker in dilated cardiomyopathy postmyocardial infarction. *J Pharmacol Exp Ther.* 2008; 325:491–499. [PubMed: 18287209]
4. Ahmet I, Morrell C, Lakatta EG, Talan MI. Therapeutic efficacy of a combination of a beta1-adrenoreceptor (AR) blocker and beta2-AR agonist in a rat model of postmyocardial infarction dilated heart failure exceeds that of a beta1-AR blocker plus angiotensin-converting enzyme inhibitor. *J Pharmacol Exp Ther.* 2009; 331:178–185. [PubMed: 19587314]
5. Talan MI, Ahmet I, Xiao RP, Lakatta EG. $\beta(2)$ AR Agonists in Treatment of Chronic Heart Failure: Long Path to Translation. *J Mol Cell Cardiol.* 2010 in print.
6. Chesley A, Ohtani S, Asai T, Xiao RP, Lunberg MS, Lakatta EG, et al. β_2 -adrenergic receptor delivers an anti-apoptotic signal to cardiac myocytes through G_i-dependent signaling pathways. *Circ Res.* 2000; 87:1172–1179. [PubMed: 11110775]
7. Zhu WZ, Zheng M, Lefkowitz RJ, Koch WJ, Kobilka B, Xiao RP. Dual modulation of cardiac cell survival and cell death by β_2 -adrenergic signaling in adult mouse heart cells. *Proc Nat Acad Sci USA.* 2001; 98:1607–1612. [PubMed: 11171998]
8. Zhu WZ, Wang SQ, Chakir K, Kolbilka BK, Cheng H, Xiao RP. Linkage of β_1 -adrenergic stimulation to apoptotic heart cell death through protein kinase A-independent activation of Ca²⁺/Calmodulin Kinase II. *J Clin Invest.* 2003; 111:617–625. [PubMed: 12618516]
9. Zheng M, Zhu W, Han Q, Xiao RP. Emerging concepts and therapeutic implications of β -adrenergic receptor subtype signaling. *Pharmacol Ther.* 2005; 509:109–115.
10. Jozwiak K, Khalid C, Tanga MJ, Berzetei-Gurske I, Jimenez L, Kozocas JA, et al. Comparative molecular field analysis of the binding of the stereoisomers of fenoterol and fenoterol derivatives to the beta2 adrenergic receptor. *J Med Chem.* 2007; 50:2903–2915. [PubMed: 17506540]
11. Beigi F, Bertucci C, Zhu W, Chakir K, Wainer IW, Xiao RP, et al. Enantioselective separation and online affinity chromatographic characterization of R,R- and S,S-fenoterol. *Chirality.* 2006; 18:822–827. [PubMed: 16917835]
12. Woo AY, Wang TB, Zeng X, Zhu W, Abernethy DR, Wainer IW, et al. Stereochemistry of an agonist determines coupling preference of beta2-adrenoceptor to different G proteins in cardiomyocytes. *Mol Pharmacol.* 2009; 75:158–165. [PubMed: 18838481]

13. Kim HS, Siluk D, Wainer IW. Quantitative determination of fenoterol and fenoterol derivatives in rat plasma using on-line immunoextraction and liquid chromatography/mass spectrometry. *J Chromatogr A*. 2009; 1216:3526–3532. [PubMed: 18778830]
14. Ahmet I, Krawczyk M, Heller P, Moon C, Lakatta EG, Talan MI. Beneficial effects of chronic pharmacological manipulation of beta-adrenoreceptor subtype signaling in rodent dilated ischemic cardiomyopathy. *Circulation*. 2004; 110:1083–1090. [PubMed: 15313944]
15. Xiao RP, Ji X, Lakatta EG. Functional coupling of the β_2 -adrenoceptor to a pertussis toxin-sensitive G protein in cardiac myocytes. *Mol Pharmacol*. 1995; 47:322–329. [PubMed: 7870040]
16. Xiao RP, Avdonin P, Zhou YY, Cheng H, Akhter SA, Eschenhagen T, et al. Coupling of β_2 -adrenoceptor to G_i proteins and its physiological relevance in murine cardiac myocytes. *Circ. Res*. 1999; 84:43–52. [PubMed: 9915773]
17. Xiao RP, Zhang SJ, Chakir K, Avdonin P, Zhu W, Bond RA, et al. Enhanced G_i signaling mediates the diminution of β_2 -adrenergic contractile response in failing spontaneous hypertensive rat heart. *Circulation*. 2003; 108:1633–1639. [PubMed: 12975249]

Figure 1.
Change of systolic blood pressure during 5 minutes of continuous intravenous infusion of normal saline, racemic fenoterol (F), RR enantiomer of racemic fenoterol (RF) or its SS-enantiomer (SR). * $p < 0.05$ vs saline group (Bofferoni correction).

Figure 2.

Results of serial echocardiography during 6 months of observation starting 2 weeks after MI induction in untreated rats (MI-nT) and rats treated with racemic fenoterol (MI-F), RR enantiomer of fenoterol (MI-RF) or SS enantiomer of fenoterol (MI-SF). End-diastolic volume (A), end-systolic volume (B), ejection fraction (C), and MI size (D). * $p < 0.05$ vs MI-nT; # $p < 0.05$ vs MI-RF and MI-SF (Bonferroni correction).

Figure 3.

MI size among animals survived to the end of observation. (A) Linear regression between MI sizes measured during the last echocardiography (Y axis) and at subsequent histological evaluation (X axis). (B) MI size in different treatment groups after conclusion of experiments.

Figure 4.

Systolic and diastolic arterial blood pressure measured monthly by tail-cuff technique in MI rats untreated or treated either with racemic fenoterol or with its enantiomers. Closed symbols are systolic blood pressure. Open symbols – diastolic.

Figure 5.

Additional control experiment. Results of serial echocardiography during 2 months of observation starting 2 weeks after MI induction in untreated rats (MI-nT) and rats treated with a double dose of RR-enantiomer of fenoterol (MI-RF2), or mixture of RR enantiomer of fenoterol and SS enantiomer of fenoterol (MI-RFSF). End-diastolic volume (A), end-systolic volume (B), ejection fraction (C), and MI size (D). * $p < 0.05$ vs MI-nT; (Bonferroni correction).

Echo-derived baseline characteristics of experimental groups prior to initiation of treatments (2 weeks after MI induction)

Table 1

Indices/Groups	Sh	MI-nT	MI-F	MI-RF	MI-SF
EDV (μL)	445±13	690±52.3 [*]	690±28.3 [*]	658±50.3 [*]	709±32.8 [*]
ESV (μL)	155±7	516±50.4 [*]	508±28.8 [*]	482±43 [*]	507±35 [*]
EF (%)	65.1±1.2	26.9±2.8 [*]	26.6±2.3 [*]	27.3±2.1 [*]	29.2±2 [*]
MI (% of LV)	NA	29.6±1.6	31.4±2	30.1±1.8	31.7±2.3

* p<0.05 vs Sh (post-hoc Bonferroni correction)

Table 2

Hemodynamic indices in Sh rats and treated and untreated rats 9 months after MI

	Sh (n=10)	MI-nT (n=11)	MI-F (n=9)	MI-RF (n=8)	MI-SF (n=9)
HR (beats/min)	304±11	301±12	284±9	333±27	272±10
SV (µl)	224±15	105±11 *	137±16 *	109±7 *	106±12 *
CO (ml/min)	67±5 *	31±4 *	38±4 *	35±2 *	29±4 *
EF (%)	51±2 *	24±3 *	24±3 *	23±1 *	29±2 *
ESP (mmHg)	103±6	99±6	92±6	98±6	92±4
EDP (mmHg)	3.7±0.4	8.8±1.9 *	5.5±1.1	9.1±1.8 *	8.0±2.6 *
Ea (mmHg/ µl)	0.47±0.03 *	1.0±0.06 *	0.77±0.08 *	0.96±0.07 *	0.95±0.08 *
(+) dP/dt (mmHg/sec)	6673±297	5076±543 *	4780±303 *	5120±329 *	4941±339 *
(-) dP/dt (mmHg/sec)	7721±531	5630±824 *	4581±318 *	5214±498 *	5500±609 *
µ (msec)	9.4±0.3	13.8±1.1 *	13.3±0.6 *	14.2±1.0 *	14.4±1.3 *
PRSW (mW/ µl)	87.8±5.1	41.6±4.0 *	28.5±8.1 *	43.8±6.4 *	40.0±5.0 *
Ees (mmHg/ µl)	0.38±0.07	0.48±0.12	0.67±0.14	0.53±0.05	0.72±0.12
Eed (10 ⁻³ mmHg/ µl)	1.3±0.2	6.0±0.9 *	4.9±0.7 *	5.0±1.1 *	7.5±1.7 *
Ea/Ees	1.5±0.2	3.0±0.5 *	1.4±0.2 #	1.9±0.1	2.0±0.5

* p<0.05 vs SH;

p<0.05 vs MI-nT (after Bonferroni correction)